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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the Application of) Examiner: Moran, Marjorie A.
Michael M. Grunstein et al.)
Serial No. 09/261,104) Art Unit: 1631
Filed: March 3, 1999) Response to Paper No.: 21
For: "COMPOSITIONS AND METHODS)
FOR TREATMENT OF ASTHMA")

DECLARATION OF MICHAEL M. GRUNSTEIN AND HAKON HAKONARSON

UNDER 37 C.F.R. §1.131

We, Michael M. Grunstein and Hakon Hakonarson, hereby
declare that:

1. We are the inventors of the invention described and claimed in U.S. Patent Application Serial No. 09/261,104, (the '104 application).
2. We have read and are familiar with the contents of the Official Action dated August 27, 2002 in the '104 application. We note that the Examiner has rejected claims 3, 9-11, 19-20, 28, and 30-42 under 35 U.S.C. §103(a) as allegedly being unpatentable over Fick et al (WO 9704807, hereafter '807 application) in view of Platz et al (US Patent No. 6,019,968, hereafter '968 patent) and Jager et al (US Patent No. 5,676,930, hereafter '930 patent). We also note that claims 4 and 14 have been rejected under 35 U.S.C. §103(a) as allegedly being unpatentable over '807 application in view of '968 patent and '930 patent and further in view of Bonnefoy et al (WO 9612741, hereafter '741 application). The purpose of this declaration is to

establish that the invention described and claimed in the '104 application (the "subject invention") was conceived of prior to April 14, 1995, which upon information and belief, predates the earliest U.S. filing dates of the '807 application and '968 patent, respectively. This declaration also establishes that the invention described and claimed in the '104 application was diligently and constructively reduced to practice at least as early as March 3, 1998, the filing date of the provisional application from which the instant application claims priority under 35 U.S.C. §119(e).

3. Conception of the invention described in '104 application is evidenced by representative flow cytometric analysis of FcεRII, FcγRII, and FcγRIII receptor proteins expression in human airway smooth muscle (ASM) tissue treated for 24 hours with human control serum or atopic asthmatic serum (Exhibits A1-A5). Data disclosed in Exhibits A1-A5 are experiments utilized in the development of methods for the treatment of asthma. It was observed that the expression of the specific IgE receptor, FcεRII, is increased in ASM tissue treated with atopic asthmatic serum (A2) compared to that in ASM tissue treated with control serum (A3). However, expression levels of the IgG receptors, FcγRII (A1) or FcγRIII receptors (A4), in ASM tissue were not affected by exposure to atopic asthmatic serum. The samples described in Exhibits A1-A4 were prepared prior to the earliest U.S. filing dates of the '807 application and '968 patent. Exhibit A5 is a composite figure of some of these data. Dates shown on Exhibit A have been masked for the purpose of this Declaration. Conception of the subject matter described in Exhibits A1-A5 occurred in the United States.
4. Attached hereto as Exhibit B1 is a copy of an abstract,

which clearly discloses Applicants' finding that both Fcγ and FcεRII receptors are expressed in ASM cells and that FcεRII receptor is up-regulated in ASM cells by exposure to atopic asthmatic serum. Also provided is Exhibit B2 which is an abstract describing further characterization of the role played by the FcεRII in the asthmatic response.

5. Attached hereto as Exhibits C1-C7 are copies of notebook pages which reveal that the effects of atopic asthmatic serum are blocked or prevented by pre-incubation of ASM with an anti-FcεRII (CD23) monoclonal antibody. C1 is a graph of the tracing data presented in Exhibits C6 and C7. The dates on the data have been masked for the purpose of this Declaration.
6. Attached hereto as Exhibits D1 and D2 are pages from our laboratory notebooks describing Southern blots showing that FcεIIR is expressed in ASM cells and that this expression is up-regulated by exposing the cells to atopic asthmatic serum. These results were observed in both human (D1) and rabbit (D2) tissue. We performed these experiments and contemporaneously recorded the data set forth in the laboratory notebook pages of Exhibit D. The dates appearing in Exhibit D have been masked for the purpose of this Declaration.
7. The results of flow cytometry analysis of FcεIIR expression are also shown in Exhibits E1-E3. ASM tissue was exposed to atopic asthmatic serum for 24 hours. The data show that relative to isotype negative control antibody (E1) FcεIIR expression was dramatically up-regulated in human asthmatic serum-treated human airway cells (E2) relative to control serum-treated cells (E3). Dates on Exhibit E are masked for

the purpose of this Declaration.

8. Exhibit F shows the results of experiments performed using ASM tissue obtained from human patients. Representative Southern blots are shown revealing that, in contrast to lung tissue, a dramatic increase in FcεRII mRNA expression is seen in the airways of asthmatic patients relative to control non-asthmatic patient airway tissue. Dates on Exhibit F have been masked for the purpose of this Declaration.
9. In order to show a reduction to practice of our invention, Exhibit G is attached hereto. Exhibit G is a copy of the provisional application filed March 10, 1998 from which the instant application claims priority under 35 U.S.C. §119(e).
10. After conceiving the invention and carrying out the experimentation described in paragraphs 3-8 above, we were diligent in reducing the invention to practice, including the period from just prior to April 14, 1995 until the invention was subsequently reduced to practice as set forth in paragraph 9 above. The Exhibits described in paragraphs 3-8 describe our work during the relevant period. Thus, we conceived of the present invention prior to the filing dates of '807 application and '968 patent and thereafter diligently reduced the claimed invention to practice.
11. All of the experiments, the results of which are presented in the Exhibits attached to this Declaration, were performed by us or under our direction and supervision.

We hereby declare that all statements made herein of our own

knowledge are true and that all statements made on information and belief are believed true; and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful statements may jeopardize the validity of the above-referenced application or any patent issued thereon.

February 27, 1963
DATE

Michael M. Grunstein
Michael M. Grunstein

DATE

Hakon Hakonarson



IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

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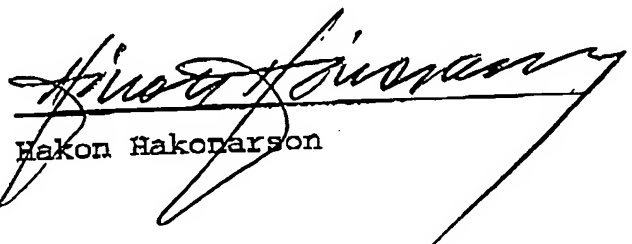
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DATE

02/27/03

DATE

Michael M. Grunstein



Hakon Hakonarson

Altered expression and action of the low-affinity IgE receptor FcεRII (CD23) in asthmatic airway smooth muscle

Hakon Hakonarson, MD, Carrie Carter, BA, Cecilia Kim, BA, and Michael M. Grunstein, MD, PhD *Philadelphia, Pa*

Background: Changes in cell surface expression of certain immunoglobulin Fc receptors have been demonstrated in leukocytes isolated from the lungs of atopic asthmatic individuals. This, together with emerging evidence that Fc receptors can also be expressed and activated in non-bone marrow-derived cell types, including airway smooth muscle (ASM), raises the hypothesis that the atopic asthmatic ASM phenotype is associated with an altered endogenous expression and action of specific Fc receptors present in the ASM itself. **Objective:** The current study addressed the above hypothesis by examining (1) whether the expression of certain key Fc receptor subtypes for IgE and IgG is altered in ASM tissue isolated from human atopic asthmatic individuals and (2) whether this altered Fc receptor expression is comparably induced in naive human ASM tissue and cultured cells after their passive sensitization with human atopic asthmatic serum or IgE immune complexes.

Methods: Messenger RNA and cell surface protein expression of the individual IgG receptor subtypes FcγRI, FcγRII, and FcγRIII, as well as the IgE receptor subtypes FcεRI and FcεRII, were examined in human ASM tissue isolated from atopic asthmatic and control (nonatopic/nonasthmatic) individuals. In addition, we examined the effects of passive sensitization of ASM tissue and cultured ASM cells with control serum, atopic asthmatic serum, or exogenously administered IgE immune complexes on Fc receptor expression and action (ie, induction of proinflammatory cytokine release).

Results: The observations demonstrate that (1) human ASM tissue expresses messenger RNA and surface protein for FcεRII, as well as for all the Fcγ receptor subtypes, (2) in contrast to unaltered Fcγ subtype expression, however, relative to control human ASM, FcεRII is significantly up-regulated in inherently asthmatic ASM tissue, (3) up-regulated expression of FcεRII represents, at least in part, an inducible phenomenon that is largely attributed to IgE immune complex-coupled

activation of the receptor, and (4) the latter action is associated with FcεRII-induced autologous elaboration of the proinflammatory cytokine, IL-1β, by the atopic sensitized ASM.

Conclusion: These observations provide new evidence that human ASM tissue expresses FcεRII in addition to all 3 subtypes of Fcγ receptors and that the expression of FcεRII is selectively increased in atopic asthmatic ASM, a phenomenon associated with IgE immune complex/FcεRII-mediated elaboration of IL-1β by the ASM itself. (*J Allergy Clin Immunol* 1999;104:575-84.)

Key words: Fc receptor expression, immunoglobulins, IL-1β, human airway smooth muscle, asthma

Exaggerated agonist-induced bronchoconstriction, attenuated β-adrenoceptor-mediated airway relaxation, and airway inflammation are all characteristic features of bronchial asthma.¹⁻³ Although the mechanism underlying these inflammation-associated changes in airway responsiveness remains largely unknown, numerous reports support a role for Fc receptor activation in both initiating and propagating the pulmonary inflammatory response in asthma.⁴⁻⁷ Thus mast cell stimulation has been implicated in mediating the immediate bronchoconstrictor response after antigen inhalation in individuals with atopic asthma, wherein IgE-coupled activation of its high-affinity receptor (ie, FcεRI) on mast cells leads to cellular degranulation and the release of various proinflammatory mediators and cytokines. The recent identification of Fc receptors on other cell types in the lung (eg, mononuclear cells, eosinophils, dendritic cells, and epithelial cells) suggests that their activation may also serve to propagate the proinflammatory airway response, most likely by the orchestrated extended release of specific cytokines.⁷⁻¹⁴ Moreover, apart from leukocytes per se, the identification of Fc receptors in resident tissue cells, including airway smooth muscle (ASM) cells,¹⁵ suggests an expanded role for Ig receptors in regulating local tissue immune responses. In this regard, we recently identified that rabbit ASM tissue and cultured ASM cells express the low-affinity receptor for IgE, FcεRII (CD23) and that activation of the latter receptor is associated with the induction of "proasthmatic" changes in agonist-mediated ASM constrictor and relaxant responsiveness.¹⁵

In light of the above evidence, the current study examined whether expression of specific Fc receptor messenger RNAs (mRNA) and proteins is altered in inherently asthmatic human ASM tissue and whether this modulated Fc receptor expression represents a potentially inducible

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Abbreviations used

ASM:	Airway smooth muscle
cDNA:	Complementary DNA
FcεRII (CD23):	Low-affinity receptor for IgE
HBSM:	Human bronchial smooth muscle
mRNA:	Messenger RNA
NaCl:	Sodium chloride
RT:	Reverse transcriptase
SFM:	Serum-free medium
SMBM:	Smooth muscle basal medium
SSC:	Standard saline citrate

phenomenon in naive ASM passively sensitized with human atopic asthmatic serum or exogenously administered IgE immune complexes. The results demonstrate that (1) human ASM tissue expresses mRNA and surface protein for the low-affinity IgE receptor FcεRII as well as for all 3 Fcγ receptor subtypes that bind to IgG, (2) in contrast to unaltered Fcγ receptor expression, relative to naive (control) human ASM, expression of FcεRII is significantly up-regulated in inherently asthmatic ASM, (3) up-regulated expression of FcεRII in human ASM is, at least in part, an inducible phenomenon, largely attributed to immune-complexed IgE/CD23-coupled interaction, and (4) the latter phenomenon is accompanied by the induced expression and elaboration of the proinflammatory cytokine IL-1β by the ASM itself. Collectively, these findings suggest a critical role for up-regulated FcεRII receptor expression and activation in mediating the proasthmatic phenotype in human ASM.

METHODS**Preparation of human ASM tissue**

Human ASM tissue (both tracheal and bronchial sections) was isolated from a 12-year-old asthmatic boy who died from status asthmaticus (patient 1). Although the patient's cardiopulmonary status responded to resuscitation, he was determined to be dead on the basis of brain perfusion scan criteria. The lung and airway tissue were freshly obtained under sterile conditions during a subsequent organ donation procedure. The ASM tissue was carefully scraped of loose connective tissue and epithelium under a microscope, prepared for RNA and protein extraction, and examined for Fc receptor expression, as described below. ASM tissue was also prepared from a 25-year-old African American woman with asthma who died from an exacerbation complicated by pneumonia (patient 2). Of note, microscopically the airway tissue specimens from both patients had all the characteristic features of asthmatic airway tissue, including the presence of ASM hypertrophy-hyperplasia. Age-matched ASM tissue from 6 individuals who had no history of asthma and who either had a lung resection for a peripheral lung carcinoma or who died from nonpulmonary related causes served as controls. The latter tissue specimens were tumor free and microscopically normal.

In extended experiments, ASM tissue from 3 of the above 6 nonasthmatic subjects was passively sensitized with either human control serum, atopic asthmatic serum, IgE alone (2–15 μg/mL), or IgE immune complexes (2–15 μg IgE, 1–5 μg/mL anti-IgE) for various time points (see below). The atopic asthmatic serum was obtained from allergic patients with moderate to severe asthma and

serum IgE levels >1000 IU/mL and 4 to 5 of 6 plus radioallergosorbent tests positive (specific IgE concentration of > 17.5 Phadebas radioallergosorbent test units/mL) to *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, and ragweed and positive skin test results to these antigens. The control serum was obtained from nonatopic nonasthmatic subjects with normal IgE levels (ie, <70 IU/mL) and negative skin test reactivity. The tissues' mRNA and protein were subsequently isolated for analysis of Fc receptor mRNA and protein expression (see below). The participation of human subjects in this study was approved by the Institutional Review Board of the Joseph Stokes, Jr Research Institute at the Children's Hospital of Philadelphia.

Preparation and sensitization of human ASM cells

To examine Fc receptor regulation in ASM cells, we used cultured human bronchial smooth muscle (HBSM) cells obtained from Clonetics (San Diego, Calif). The HBSM cells were derived from 2 healthy male donors aged 16 and 21 years. The cells were fully characterized with specific markers to confirm their selective smooth muscle phenotype and to exclude contamination with other cell types, according to the manufacturer's protocol. The cells were grown in smooth muscle basal medium (SMBM) supplemented with 5% FBS, insulin (5 ng/mL), epithelial growth factor (10 ng/mL, human recombinant), fibroblast growth factor (2 ng/mL, human recombinant), gentamicin (50 ng/mL), and amphotericin B (50 ng/mL). The standard experimental protocol involved growing the cells to confluence in the above medium. The cells were then starved in unsupplemented SMBM (SFM) for 24 hours, at which time the cells were treated with either human control serum from nonatopic-nonasthmatic subjects or serum from subjects with atopic asthma in the absence and presence (40 μg/mL) of an anti-CD23 monoclonal blocking antibody (anti-CD23 mAb). In other experiments cells were treated with SFM alone or SFM in the presence of maximum effective concentrations of IgE or IgE immune complexes. The cells were then examined for Fc receptor mRNA and protein expression, as described below.

Determination of Fcγ and Fcε receptor mRNA expression

Total RNA was isolated from the above tissue and cell preparations with use of the modified guanidinium thiocyanate-phenol-chloroform extraction method to include proteinase K (in 5% SDS) digestion of protein in the initial RNA pellet.¹⁶ The concentration of each RNA sample was then determined spectrophotometrically. This procedure consistently produced yields of 15 to 25 μg of intact RNA per each tissue specimen under study or T-75 flask of HBSM cells. To analyze for mRNA expression of the FcγRI, FcγRII, FcγRIII, FcεRI, and FcεRII receptor subtypes, we used a reverse transcriptase (RT)-PCR protocol and human specific primers for these Fc receptor subtypes. Complementary DNA (cDNA) was synthesized from total RNA isolated from (1) naive versus inherently atopic asthmatic human ASM tissue and (2) human ASM tissue or ASM cells passively sensitized with either IgE alone, IgE immune complexes, human control serum, or atopic asthmatic serum in the absence and presence of anti-CD23 mAb. cDNA from activated peripheral blood mononuclear cells served as positive controls for the individual Fcγ and Fcε receptor expression analyses. The cDNA was primed with oligodeoxythymidine 12–18 and extended with Superscript II reverse transcriptase (Gibco BRL). PCR was used to amplify Fc receptor-specific products from each cDNA reaction with use of the following Fc receptor primer pairs, on the basis of published sequences of the human FcγRI, FcγRII, FcγRIII, and FcεRI and FcεRII genes,^{17,18} and included the following primer sets:

FcγRIa:

5'-Primer 5'-ATGTGGTTCTTGACAACTCTGCTC-3'
3'-Primer 5'-ATGTCTGTCTTCTTGAAGGCTGGA-3'
Product is 1038 bp

FcγRIIa,c:

5'-Primer 5'-GACTCCATTCACTGGTTCCA-3'
3'-Primer 5'-GTCAGCTGTTTCATAGTCATTG-3'
Product is 644 bp

FcγRIII:

5'-Primer 5'-AAGATCTCCCAAAGGCTGTG-3'
3'-Primer 5'-ATGGACTTCTAGCTGCACCG-3'
Product is 254 bp

FcεRI:

5'-Primer 5'-GGAACGGGAATTACCATCCT-3'
3'-Primer 5'-AAAGACGATCATCGGGAACC-3'
Product is 313 bp

FcεRII:

5'-Primer 5'-CGTCTCTCAAGTTTCCAAG-3'
3'-Primer 5'-GCACTTCCGTTGGAATTG-3'
Product is 333 bp

RPL7:

5'-Primer 5'-AAGAGGCTCTCATTTTCCTGGCTG-3'
3'-Primer 5'-TCCGTTCCCTCCCCATAATGTTCC-3'
Product is 157 bp

The cycling profile used was as follows: denaturation 95°C for 1 minute, annealing 52°C to 62°C for 1.0 to 1.5 minutes, and extension 72°C for 1 to 2 minutes and 34 to 40 cycles for the FcγRI, FcγRII, FcγRIII, FcεRI and FcεRII receptor genes and 26 cycles for the RPL7 gene. The number of cycles was determined to be in the linear range of the PCR products. The PCR reactions for the human Fcγ, Fcε, and RPL7 primers were performed with use of equivalent amounts of cDNA prepared from 2.5 μg of total RNA. Equal aliquots of each PCR reaction were then run on a 1.2% agarose gel and subsequently transferred to a zeta-probe membrane overnight in 0.4N sodium hydroxide. After capillary transfer, the DNA was immobilized by UV cross-linking with use of a Stratilinker UV Crosslinker 2400 at 120,000 μJ/cm² (Stratagene). Prehybridization in a Techne hybridization oven was conducted for 2 to 3 hours at 42°C in 50% formaldehyde, 7% (wt/vol) SDS, 0.25 mol/L sodium chloride (NaCl), 0.12 mol/L sodium phosphate (pH 7.2), and 1 mmol/L EDTA. Hybridization was for 20 hours at 42°C in the same solution. The FcγRI, FcγRII, FcγRIII, FcεRI, FcεRII, and RPL7 DNA levels were assayed by Southern blot analysis with phosphorus 32-labeled probes. These probes were prepared by pooling several RT-PCR reactions for the individual Fc and RPL7 PCR fragments and purifying them from a 1.2% agarose gel with use of the Qiaex II agarose gel extraction kit. The individual Fc receptor cDNA fragments were subsequently sequenced for product confirmation. Washes were as follows: 1 for 15 minutes in 2× standard saline citrate (SSC), 0.1% SDS at room temperature; 1 for 15 minutes in 0.1× SSC, 0.1% SDS at room temperature; and 2 for 15 minutes at 50°C in 0.1× SSC, 0.1% SDS. Southern blots were quantitated by direct measurements of radioactivity in each band with use of a PhosphorImager (Molecular Dynamics).

Fifteen micrograms of cytoplasmic RNA pertaining to each time point was also fractionated in 1% agarose, formaldehyde denaturing gels. After capillary transfer, cross-linking, and prehybridization, the levels of mRNA were analyzed with use of the above ³²P-labeled cDNA fragments for the individual Fc receptor genes prepared by random priming, as previously described in our laboratory.¹⁹ Northern blots were quantitated by direct measurement of radioactivity in each band with use of the PhosphorImager.

Determination of Fcγ and Fcε receptor protein expression

Expression of cell surface protein for the FcγRI, FcγRII, FcγRIII, and FcεRII receptors was assayed by Western blot analysis of membrane protein samples isolated from naive (control) and human asthmatic ASM tissue. The ASM was then minced and homogenized in 50 mmol/L TRIS-hydrochloric acid, 150 mmol/L NaCl, 1 mmol/L EDTA (pH 7.4) containing 1 mmol/L phenylmethylsulfonyl fluoride, 5 μg/mL aprotinin, and 5 μg/mL leupeptin. Nuclei and large particulates were removed by centrifugation at 100g for 5 minutes. The supernatant was then centrifuged at 100,000g for 1 hour to pellet the membrane fractions. The membrane pellet was resuspended in the same Tris-EDTA buffer, and the protein concentration was measured with the Lowry assay. Equivalent amounts (30-50 μg) of membrane protein were fractionated in 11% SDS-polyacrylamide gels followed by transfer to nitrocellulose membranes. The membranes were then blotted overnight at room temperature in 25 mmol/L TRIS-hydrochloric acid (pH 7.5), 150 mmol/L NaCl, and 0.05% Tergitol NP-40 containing 5% nonfat milk, as previously described in our laboratory.²⁰ The primary mouse antihuman FcγRI, FcγRII, FcγRIII, and FcεRII antibodies used were diluted 1:250 to 1:500 and incubated for 1 hour at room temperature. All primary and secondary antibody incubations and washes were done in 25 mmol/L TRIS-hydrochloric acid (pH 7.5), 150 mmol/L NaCl, and 0.05% NP-40 containing 0.50% nonfat milk. The individual Fc receptor levels were detected with enhanced chemiluminescence after a 1-hour incubation with a 1:1000 dilution of an antimouse horseradish peroxidase-linked secondary antibody and subsequent exposure to autoradiography film. Expression levels of the individual Fc receptor protein were quantitated with use of laser densitometry (Bio-Rad, Hercules, Calif).

Fc receptor cell surface expression was also examined in human ASM cells by flow cytometric analysis with a Coulter EPICS Elite flow cytometer (Coulter EPICS Division, Hialeah, Fla) equipped with a 5-W argon laser operated at 488 nmol/L and 300 mW output. Fluorescence signals were accumulated as 2-parameter fluorescence histograms with both percent positive cells and mean channel fluorescence being recorded. Cells treated for 24 hours with either 20% human control serum or 20% human atopic asthmatic serum were resuspended in buffer, dispersed by passage through a 23-gauge needle, and then separately stained with primary mouse antihuman antibodies specific for the individual FcγRI, FcγRII, FcγRIII, FcεRI, and FcεRII receptor genes (Serotec, Raleigh, NC) in 1:250 to 1:500 dilution. After subsequent repeat washing, FITC-labeled F(ab')₂ goat antimouse IgE and IgG fragments were used as secondary antibodies and incubated for 1 hour in 1:500 to 1:1000 dilutions in PBS containing 0.5% BSA. Activated peripheral blood lymphocytes isolated from blood donors were used as the positive controls for the Fc receptor expression assays. The cells were also stained with mouse antibodies of the identical isotypes as the Fc receptor mAbs to measure background fluorescence (ie, mouse IgG1 negative control). The antibody-stained cells were then evaluated by flow cytometry and analyzed with use of Elite Immuno 4 statistical software (Coulter EPICS Division). Fluorescence intensities were expressed as percent positive cells, including mean channel fluorescence.

Finally, an immunofluorescence detection assay was also used to examine for FcεRII surface receptor expression in ASM cells after a 24-hour treatment with human control versus atopic asthmatic serum, as described above. After standard preparation, the ASM cells were fixed in acetone and then mounted on poly-L-lysine-coated slides. The cells were incubated with PBS buffer containing 10% human serum to suppress nonspecific staining and subsequently labeled overnight at 4°C with primary mouse antihuman CD23 anti-

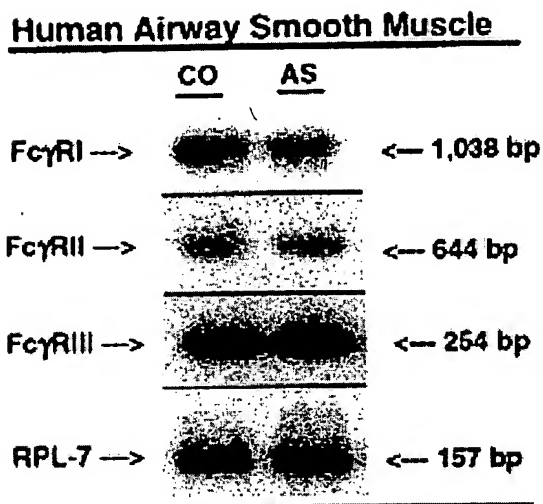


FIG 1. Comparison of expression of Fc γ RI (1038 bp), Fc γ RII (644 bp), and Fc γ RIII (254 bp) receptor mRNAs with use of RT-PCR in control (CO) and inherently asthmatic (AS) human ASM tissue. Expression of RPL7 was used to control for loading. Blots were probed with human specific Fc γ RI, Fc γ RII, and Fc γ RIII 32 P-labeled cDNA probes. Note: Human ASM tissue expresses mRNA for both the high-affinity Fc γ RI and the low-affinity Fc γ RII and Fc γ RIII receptors, as determined by expected sizes of respective Fc γ RI, Fc γ RII, and Fc γ RIII cDNA fragments, and there were no differences in mRNA expression signals between control and asthmatic ASM. Data are representative of 3 separate experiments with ASM tissue isolated from patient 1. Comparable results were obtained with ASM tissue isolated from patient 2.

body in 1:250 dilution. In control sections the primary antibody was replaced by IgG of the same isotype according to the manufacturer's protocol (mouse IgG1 negative control). Parallel cell slides were also stained with an α -actin antibody to exclude contamination of the ASM cells with non-actin-containing cells. After subsequent repeat washing, FITC-labeled F(ab') $_2$ goat antimouse IgE or IgG fragments were used as secondary antibodies, as described above. After serial washing, the slides were examined under a fluorescent microscope, and quantitative analysis of the protein localization in the cell and tissue sections under the above experimental conditions was performed with use of the Metamorph Imaging System interfaced with a Nikon Diaphot 300 Image Analyzer that uses a Hamamatsu CCD camera.

ELISA measurements of IL-1 β protein

IL-1 β protein levels were measured in the culture media from microscopically isolated human ASM tissues at baseline and after a 24-hour exposure to maximum effective concentrations of either IgE alone, IgE immune complexes, human control serum, or human atopic asthmatic serum in the absence and presence of anti-CD23 mAb. IL-1 β protein was quantitatively assessed with an enzyme-specific immunoassay, as previously described.¹⁹ The latter assay was performed with a double-antibody sandwich strategy in which an acetylcholine esterase, Fab-conjugated IL-1 β -specific secondary antibody was targeted to a first cytokine-captured antibody. The enzymatic activity of the acetylcholine esterase was measured spectrophotometrically and, relative to a linear standard curve (range 0-250 pg/mL), the results were used to quantify the amount of the targeted IL-1 β present in the tissue culture media.

Statistical analysis

Statistical analysis was performed by means of the 2-tailed paired Student *t* test. *P* values <0.05 were considered significant.

Reagents

The human airway tissue was provided by the Cooperative Human Tissue Network, which is funded by the National Cancer Institute. The human ASM cells and SMBM medium were obtained from Clonetics, Walkersville, Md. The Fc γ RI, Fc γ RIIAC, Fc γ RIIB, Fc γ RIII, Fc ϵ RI, Fc ϵ RII, and RPL7 primers were obtained from Integrated DNA Technologies, Coralville, Ia. The human myeloma IgE and the goat-antihuman IgE antibodies were purchased from Biodesign, Kennebunk, Me. The Fc γ RI, Fc γ RII, Fc γ RIII, and Fc ϵ RII mAbs and the F(ab') $_2$ -FITC fragments used in protein assay studies were purchased from Serotec, Raleigh, NC. The anti-CD23 monoclonal blocking antibody (mAb135) was the generous gift of Dr M. Sarfati, Montreal, Quebec, Canada.

RESULTS

Fc receptor mRNA expression in inherently asthmatic and atopic asthmatic serum-sensitized ASM

To examine the expression of different Fc receptors in human ASM tissue and whether this expression is altered in inherently atopic asthmatic ASM, we used RT-PCR incorporating Fc receptor-specific primers for the different Fc γ and Fc ϵ receptor subtypes. Southern blots were then generated (see Methods) and probed with human cDNA probes specific for the individual Fc γ and Fc ϵ receptor genes. A 157-bp RPL7 probe was also used to control for gel loading, and the signals for the Fc γ , Fc ϵ , and RPL7 PCR products were quantitated on a Phosphor-Imager. As depicted in Fig 1, ASM tissue expressed mRNA for all 3 Fc γ receptor subtypes and there were no significant difference in the magnitudes of the signals for either Fc γ RI, Fc γ RII, or Fc γ RIII between control (nonatopic nonasthmatic) and inherently asthmatic ASM. In contrast to these observations, expression of the high-affinity IgE receptor, Fc ϵ RI, was undetectable in either the control or asthmatic ASM tissue specimens (Fig 2). mRNA expression of the low-affinity IgE receptor, Fc ϵ RII (ie, CD23), however, was present in both the control and asthmatic ASM tissues and, relative to the control ASM, the Fc ϵ RII signal was markedly up-regulated in the asthmatic ASM (Fig 2).

To determine whether the above selectively up-regulated expression of Fc ϵ RII mRNA in the inherently atopic asthmatic ASM tissues potentially represented an inducible phenomenon, similar to that recently reported in rabbit ASM passively sensitized with human atopic asthmatic serum,¹⁵ comparable experiments were conducted on adjacent segments of human control (nonatopic nonasthmatic) ASM tissue sections that were passively sensitized for 24 hours with either human control or atopic asthmatic serum. Subsequent Southern blot analysis demonstrated that, although the Fc ϵ RII signal was detectable in the control serum-treated tissues, expression of Fc ϵ RII was markedly enhanced in the atopic asthmatic serum-sensitized ASM. In contrast, the

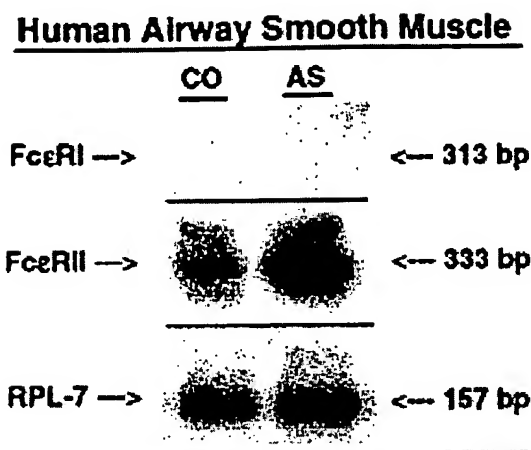


FIG 2. Comparison of expression of FcεRI (313 bp) and FcεRII (333 bp) receptor mRNAs with RT-PCR in control (CO) and inherently asthmatic (AS) human ASM tissue. Expression of RPL7 was used to control for loading. Blots were probed with human specific FcεRI and FcεRII ³²P-labeled cDNA probes. Note: In contrast to undetectable expression of FcεRI, human ASM tissue expresses mRNA for low-affinity FcεRII receptor, as determined by expected size of its FcεRII cDNA fragment, and expression of FcεRII mRNA signal is markedly increased in inherently asthmatic human ASM. Data are representative of 3 separate experiments with ASM tissue isolated from patient 1. Comparable results were obtained with ASM tissue isolated from patient 2.

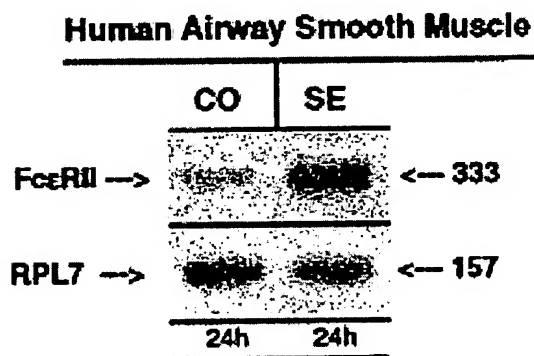


FIG 3. Representative Southern blot (n = 4) prepared from paired human ASM tissue samples incubated with control (CO) or atopic asthmatic (SE) serum for 24 hours. PCR products were amplified with human-specific FcεRII and RPL7 primers and probed with human-specific 333-bp FcεRII and 157-bp RPL7 human ³²P-labeled cDNA probes. Note: Marked induction of FcεRII expression in atopic asthmatic serum-sensitized sample.

constitutively expressed RPL7 signal was similar in both tissue preparations (Fig 3).

In light of the above results, together with our earlier pharmacodynamic evidence demonstrating a critical role for immune-complexed IgE/CD23 interaction in mediating proasthmatic changes in agonist responsiveness in atopic asthmatic serum-sensitized rabbit ASM tissues,¹⁵ we next examined whether our observed up-regulation of FcεRII expression in the atopic asthmatic serum-sensitized

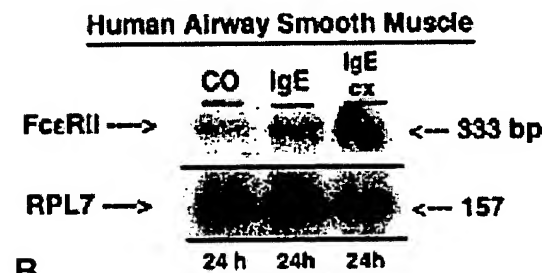
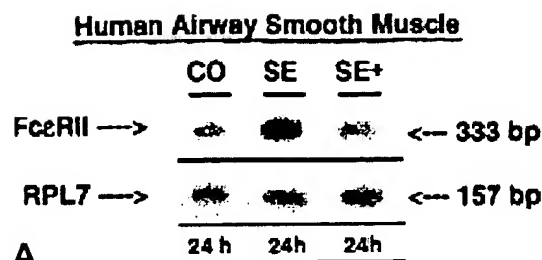


FIG 4. A, Comparison of expression of FcεRII mRNA in human ASM tissue after 24-hour treatment with either control (CO) or atopic asthmatic serum in absence (SE) and presence (SE+) of anti-CD23 mAb. **B,** Comparison of FcεRII mRNA expression in human ASM treated for 24 hours with vehicle alone (CO) or maximal effective concentrations of IgE alone or IgE immune complexes (IgE cx). Products of RT-PCR reactions with human-specific primers for FcεRII receptor mRNA are shown. Expression of RPL7 was used to control for gel loading. Blots were probed with above ³²P-labeled human-specific FcεRII and RPL7 cDNA probes. Note: Anti-CD23 mAb significantly attenuated induction of FcεRII mRNA expression in atopic asthmatic-serum sensitized ASM (**A**). Comparably, treatment of human ASM with IgE immune complexes also significantly induced enhanced mRNA expression of FcεRII, whereas IgE alone had a lesser effect (**B**). Data are representative of 4 separate experiments.

state was associated with IgE-coupled activation of the FcεRII receptor itself. With use of ASM tissue sections microscopically isolated from the same human tracheal and bronchial specimens (see Methods) we found that this up-regulation of FcεRII expression was largely prevented by pretreating the atopic serum-sensitized tissues with a monoclonal anti-CD23 blocking antibody (SE+) (Fig 4, A), whereas the tissue samples exposed to atopic asthmatic serum (SE) displayed markedly induced FcεRII expression relative to the control (CO) serum-treated samples. Moreover, in complementary experiments examining the effects of exogenous administration of either human IgE alone or IgE immune complexes to naive ASM tissues, we found that treatment of the tissues for 24 hours with IgE immune complexes induced a pronounced up-regulated expression of FcεRII mRNA, whereas the corresponding RPL7 signal was unaffected (Fig 4, B). Comparably, exogenous administration of IgE alone had a qualitatively similar effect on FcεRII expression; however, the magnitude of this effect was relatively modest.

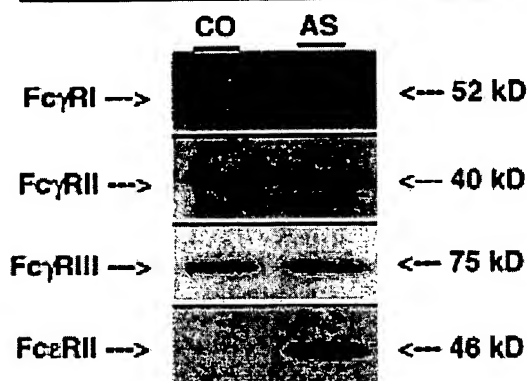
Human Airway Smooth Muscle

FIG 5. Representative Western blots depicting Fc γ RI (~52,000 kD), Fc γ RII (~40,000 kD), Fc γ RIII (~75,000 kD), and Fc ϵ RII (~46,000 kD) expression in membrane homogenates from human control (CO) and inherently asthmatic (AS) human ASM tissue. Fifty micrograms of membrane protein was loaded in each lane. Note: In contrast to unaltered Fc γ RI, Fc γ RII and Fc γ RIII expression, relative to control ASM, expression of Fc ϵ RII protein was significantly enhanced in inherently asthmatic ASM. Data are representative of 3 separate experiments with ASM tissue isolated from patient 1. Comparable results were obtained with ASM tissue isolated from patient 2.

Fc receptor protein expression in inherently asthmatic and atopic asthmatic serum-sensitized ASM

Cell surface expression of the Fc receptors Fc γ RI, Fc γ RII, Fc γ RIII, and Fc ϵ RII was also examined with use of Western immunoblot analysis of membrane protein samples isolated from human naive (control) and inherently asthmatic ASM tissues (see Methods). As demonstrated in Fig 5, protein expression signals were detected for the Fc γ RI, Fc γ RII, and Fc γ RIII receptors, and there were no discernible differences in these signals between the control and asthmatic ASM tissues. In contrast, although Fc ϵ RII protein expression was virtually undetectable in the control ASM, the expression of Fc ϵ RII was significantly up-regulated in the asthmatic ASM (Fig 5).

Given the above observations, in extended flow cytometric experiments we further examined whether cultured human ASM cells also express cell surface proteins for the Fc γ RI, Fc γ RII, Fc γ RIII, and Fc ϵ RII receptor subtypes and whether this expression is altered after passive sensitization of the cells with atopic asthmatic serum. As shown in Fig 6, ASM cells expressed surface proteins for the above Fc receptor subtypes (Fig 6, A), and there were no differences in the expression signals for the Fc γ receptors between cells that were exposed to control versus atopic asthmatic serum. In contrast to unaltered Fc γ receptor expression, relative to control cells, 24-hour exposure of the ASM cells to atopic asthmatic serum induced up-regulated cell surface expression of the Fc ϵ RII receptor (Fig 6, B). Finally, to further substantiate the latter observations, we also examined for altered Fc ϵ RII receptor expression in atopic asthmatic serum-

sensitized human ASM cells by immunofluorescence staining (see Methods). As shown in Fig 7, relative to the isotype-negative control antibody (upper panels), staining with a fluorescent-labeled anti-CD23 antibody was weakly positive in the control serum-treated cells (left panel). Comparatively, in accordance with the above flow cytometric analysis, immunofluorescence staining for the CD23 receptor protein was notably enhanced in the atopic asthmatic serum-sensitized ASM cells (right panel).

Taken together, the above observations demonstrate that (1) human ASM expresses the mRNAs and proteins for the Fc γ RI, Fc γ RII, and Fc γ RIII receptors for IgG, as well as for the Fc ϵ RII receptor for IgE, (2) relative to control ASM, the expression of Fc ϵ RII is selectively up-regulated in asthmatic ASM, and (3) up-regulated Fc ϵ RII receptor expression can be induced in naive ASM after exposure of the tissue or cultured cells to either human atopic asthmatic serum or exogenously administered IgE immune complexes.

Release of IL-1 β by atopic asthmatic sensitized ASM

We recently reported that IL-1 β protein is elaborated by rabbit ASM tissue or cultured cells passively sensitized with human atopic asthmatic serum.¹⁹ To the extent that the latter release of IL-1 β was found to act in an autocrine fashion to endogenously induce proasthmatic changes in ASM responsiveness,^{19,21} we next examined whether IgE immune complex-treated or atopic asthmatic serum-sensitized human ASM depicts altered IL-1 β protein release. The latter was analyzed with use of an enzyme-specific immunoassay (see Methods). There were no detectable levels of IL-1 β protein in the culture media of ASM tissues exposed for 24 hours to vehicle or IgE alone. In contrast, tissues that were exposed to immune-complexed IgE (Fig 8, A) or atopic asthmatic serum (Fig 8, B) depicted markedly enhanced elaboration of IL-1 β protein into the culture media at 8 and 24 hours. Moreover, this induced release of IL-1 β was inhibited by pretreating the atopic serum-sensitized tissues with an anti-CD23 monoclonal blocking antibody (Fig 8, B), implicating a role for Fc ϵ RII activation in mediating the release of IL-1 β in the sensitized state.

DISCUSSION

Bronchial asthma is characterized by exaggerated agonist-induced bronchoconstriction, attenuated β -adrenoceptor-mediated airway relaxation and airway inflammation, the latter most notably involving infiltration of the airways with eosinophils and lymphocytes.^{11,12,22} Although the mechanistic interplay between inflammation and the associated changes in airway responsiveness remains to be identified, it is relevant to note that the altered airway responsiveness in asthmatic individuals has been associated with elevations in total and antigen-specific serum IgE.^{23,24} Moreover, genetic analyses of family members with allergic asthma have demonstrated a significant correlation between altered airway respon-

Human Airway Smooth Muscle Cells

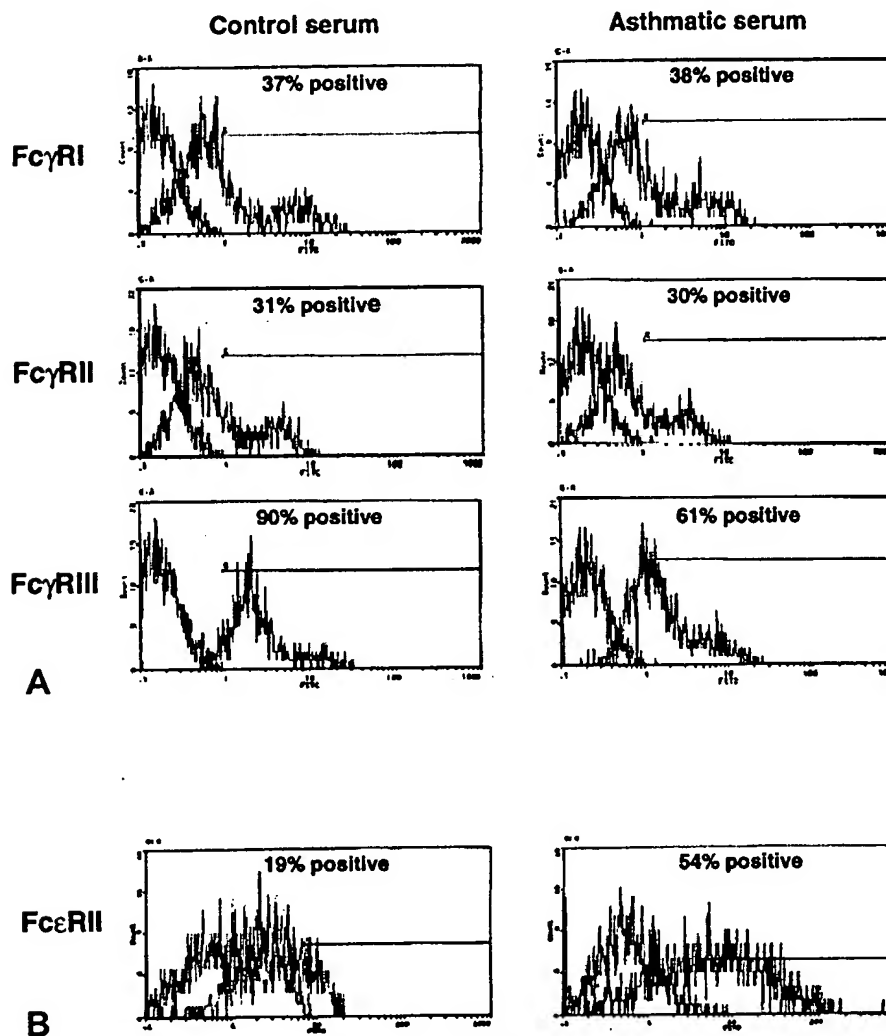


FIG 6. Representative flow cytometric analysis of FcγRI, FcγRII, and FcγRIII expression (A) and FcεRII cell surface protein expression (B) in human ASM cells treated for 24 hours with human control serum (left) and human atopic asthmatic serum (right). The ASM cells were stained with mouse antihuman mAbs specific for the individual FcγRI, FcγRII, FcγRIII, and FcεRII receptors. Levels of nonspecific background staining were measured by staining with isotype control IgG antibody (left histogram in each panel). Goat antimouse FITC-conjugated secondary antibody was used for detection of the signals. Note: Relative to cells exposed to control serum, there was significantly induced enhanced cell surface staining for FcεRII in cells that were exposed to atopic asthmatic serum ($P < .01$, $n = 3$). In contrast, atopic asthmatic serum had no appreciable effect on FcγRI, FcγRII, or FcγRIII expression.

siveness and serum IgE levels.^{25,26} As a first approximation, these findings suggest a mechanistic relationship between serum IgE levels and changes in airway responsiveness in atopic asthma. Indeed, it is well established that IgE can induce an immediate hypersensitivity reaction in the airways after allergen exposure and that this acute bronchoconstriction is initiated by degranulation of mast cells after activation of their high-affinity IgE receptor, FcεRI.⁴⁻⁷ Furthermore, progression of the ini-

tial IgE/FcεRI-coupled mast cell response into the late-phase asthmatic response has been largely attributed to the orchestrated extended release of various cytokines by other stimulated infiltrating inflammatory cell types.^{5,10,11,14} In this regard, it has been recently demonstrated that expression of the inducible form of the low-affinity IgE receptor (FcεRII) is up-regulated on monocytes and lung alveolar macrophages,^{27,28} as well as on circulating B lymphocytes²⁹⁻³¹ isolated from atopic asth-

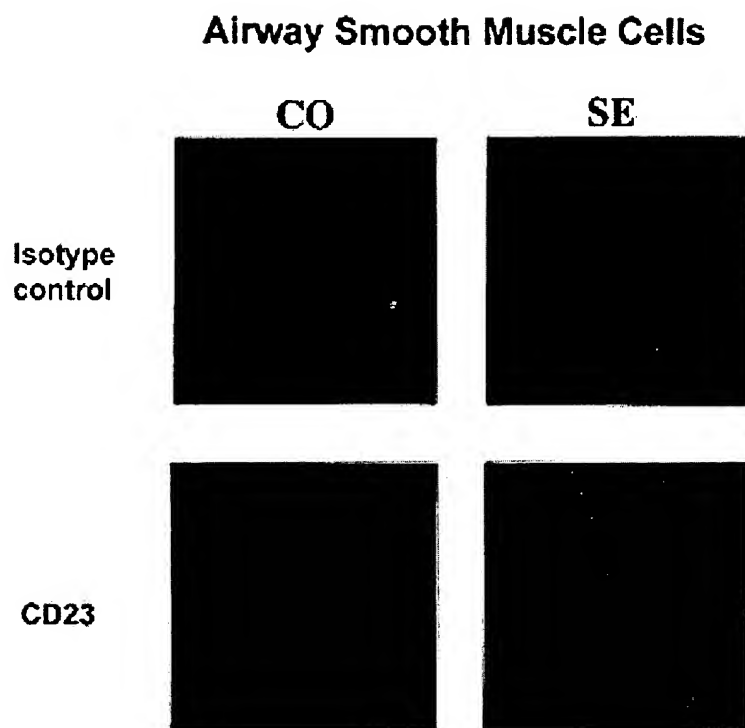


FIG 7. Representative immunofluorescence staining ($n = 4$) for CD23 surface receptor protein in control serum-treated (left) and atopic asthmatic serum-sensitized (right) ASM cells. (Original magnification $\times 100$). F(ab')₂-FITC conjugated fragments were used to detect the primary monoclonal anti-CD23 antibody. Isotype-negative control antibody was used to control for nonspecific staining (upper). Note: Significantly enhanced FcεRII (CD23) receptor staining in ASM cells that were treated with atopic asthmatic serum.

matic subjects. Thus, apart from mast cells per se, IgE-Fcε receptor interactions involving other bone marrow-derived proinflammatory cells may also contribute to the allergic asthmatic pulmonary response.

Notwithstanding the above considerations, we recently identified the presence of Fc receptors in ASM tissue and cultured ASM cells and found that specific activation of FcεRII in rabbit ASM was associated with the induction of proasthmatic changes in agonist-mediated ASM constrictor and relaxant responsiveness.¹⁵ In light of these findings, the current study investigated whether expression of specific Fc receptors is altered in inherently asthmatic human ASM tissue and whether such altered Fc receptor expression represents a potentially inducible phenomenon in naive human ASM that is passively sensitized with human atopic asthmatic serum or IgE immune complexes. The results provided compelling new evidence demonstrating that (1) although Fcγ receptor subtype expression is present and similar in both isolated human control and inherently asthmatic ASM, expression of the low-affinity receptor for IgE (FcεRII) is relatively markedly up-regulated in the asthmatic tissue, (2) qualitatively comparable selective up-regulation of FcεRII expression is inducible in naive (control) ASM passively sensitized with human atopic asthmatic serum or IgE immune complexes, and (3) the latter inducible

phenomenon is associated with an autologous FcεRII-coupled release of the proinflammatory cytokine IL-1β by the sensitized ASM. In light of these findings, the concept is raised that, apart from an implied role(s) for infiltrating leukocytes, the autologously up-regulated expression of FcεRII in ASM may also serve to foster the proasthmatic phenotype. Indeed, in extended support of this concept, we recently reported that the induced, IL-1β-dependent, proasthmatic changes in constrictor and relaxant responsiveness in rabbit ASM passively sensitized with human atopic asthmatic serum or IgE immune complexes is preventable by pretreating the sensitized ASM with a monoclonal anti-CD23 blocking antibody.¹⁵

In addressing the mechanism underlying our observed increased expression of FcεRII in inherently atopic asthmatic ASM, our extended observations suggest that this may represent an inducible phenomenon in naive human ASM. Indeed, in agreement with our recent findings using rabbit ASM,¹⁵ we found that either exposure of naive human ASM tissue to atopic asthmatic serum or IgE immune complexes induced comparably increased FcεRII receptor expression in the sensitized ASM. Moreover, our findings demonstrated that this experimentally induced up-regulated expression of FcεRII was largely prevented by pretreating the human ASM tissue with an anti-CD23 blocking antibody (Fig 4, A and B). Thus our

findings support the presence of an IgE/CD23-dependent autocrine mechanism that serves to autologously up-regulate FcεRII expression in sensitized human ASM. This concept is in accord with a number of earlier studies that have reported a direct correlation between serum IgE levels and FcεRII and FcεRI expression in other cell types.³²⁻³⁴ Although these reports raised the notion of a possible common mechanism regulating the production of both IgE and its receptors, more recent evidence based on studies with different cell lines suggests that IgE itself may act to enhance FcεRI^{35,36} or FcεRII expression.³⁷⁻³⁹ Our current observations concur with the latter notion and, accordingly, suggest the presence of a positive feedback system in human ASM wherein IgE up-regulates the expression of its own low-affinity receptor.

In previously examining the mechanistic link between exposure of naive rabbit ASM tissue to atopic asthmatic serum and its resultant altered agonist responsiveness, with the same experimental model described here, we found that the induced proasthmatic changes in ASM responsiveness were initiated by IgE/FcεRII-coupled activation, associated with the induced autologous expression and autocrine action of IL-1β in the atopic sensitized ASM.^{15,19} Moreover, the mechanism of action of IL-1β in eliciting the proasthmatic changes in ASM responsiveness was attributed to its induced up-regulated expression of Gi protein, specifically Giα₂ and Giα₃, which inhibit intracellular cyclic AMP accumulation.²¹ Our current study did not allow for comparable examination of the above mechanism of altered responsiveness in human ASM tissue because of its limited availability in quantity sufficient for pharmacodynamic experiments. Nevertheless, consistent with the mechanism identified in rabbit ASM,¹⁵ we found that human ASM tissue passively sensitized with atopic asthmatic serum or IgE immune complexes depicts markedly enhanced release of IL-1β protein into the culture medium (Fig 8, A). In addition, we found that the latter induced release of IL-1β was largely prevented by pretreating the sensitized human ASM with an anti-CD23 blocking antibody (Fig 8, B). Thus, as a first approximation, it appears reasonable to suspect that sensitized human ASM tissue may undergo proasthmatic changes in its responsiveness that are comparable to those seen in sensitized rabbit ASM. This possibility, however, remains to be experimentally addressed.

In conclusion, the current study examined the presence and regulation of Fc receptors in inherently asthmatic human ASM tissue and in naive human ASM passively sensitized with human atopic asthmatic serum and IgE immune complexes. The results demonstrate that (1) human ASM tissue expresses mRNA and surface protein for the low affinity receptor for IgE, FcεRII, as well as for the FcγRI, FcγRII, and FcγRIII receptor subtypes that bind to IgG, whereas expression of the high-affinity receptor for IgE (FcεRI) is undetectable, (2) in contrast to unaltered Fcγ subtype expression, relative to naive (control) human ASM, expression of FcεRII mRNA and protein is significantly up-regulated in inherently asth-

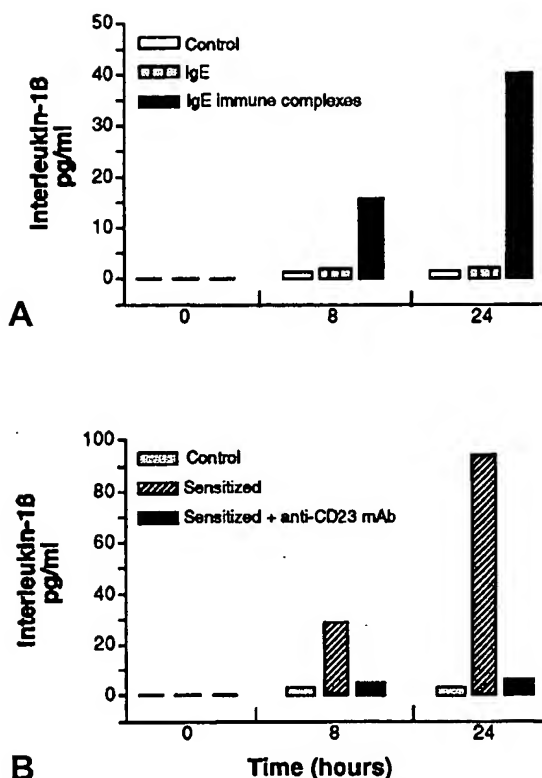


FIG 8. Comparison of mean levels of IL-1β protein released into culture media of ASM tissues after 0-, 8-, and 24-hour exposure to (A) SFM alone (open bars), IgE (hatched bars), or IgE immune complexes (filled bars) and (B) human control serum (open bars) or human atopic asthmatic serum in absence (hatched bars) and presence (filled bars) of anti-CD23 mAb. Note: Human ASM tissue exposed to IgE immune complexes or to atopic asthmatic serum released significantly increased ($P < .001$, $n = 5$) levels of IL-1β protein into tissue culture medium, whereas treatment with serum-free medium, human control serum, or IgE alone had no appreciable effect. Moreover, this effect of atopic asthmatic serum was largely prevented by pretreatment with anti-CD23 mAb. Data are representative of 3 separate experiments.

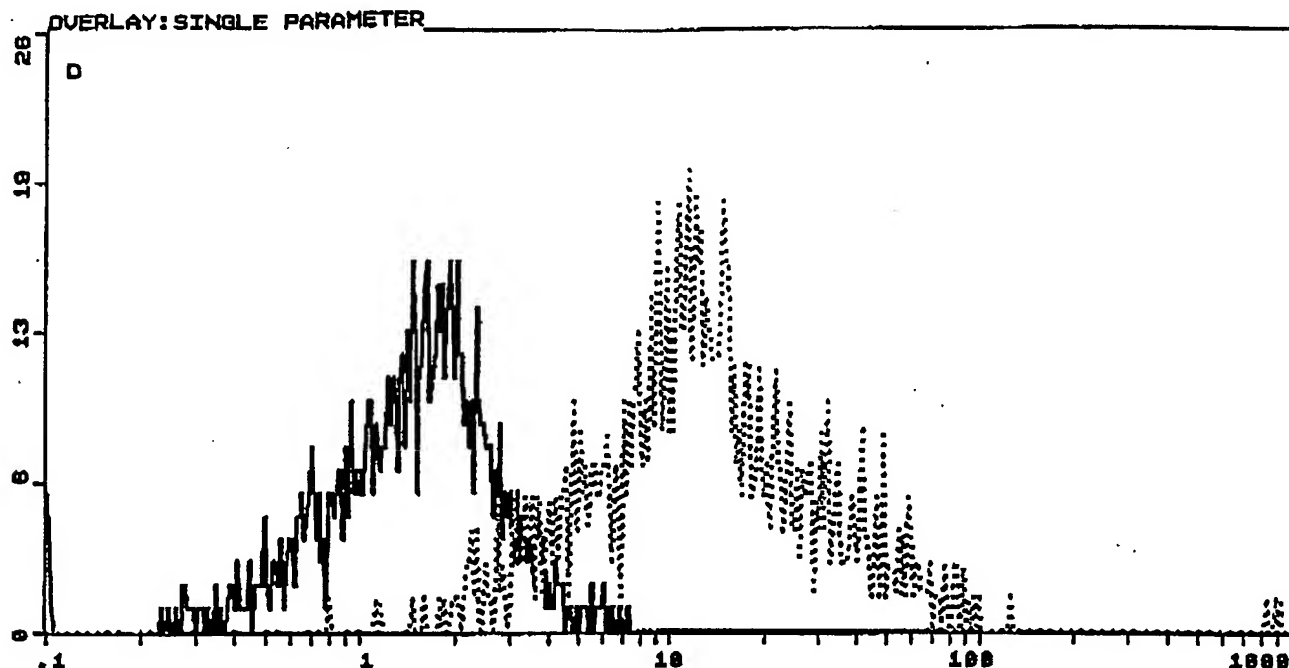
matic ASM tissues, (3) up-regulated expression of FcεRII represents, at least in part, an inducible phenomenon that is largely attributed to IgE-coupled activation of the FcεRII receptor itself, and (4) the latter action is associated with FcεRII-induced autologous elaboration of IL-1β protein by the sensitized ASM. Although comparably up-regulated expression of the FcεRII receptor has been demonstrated in different bone marrow-derived circulating leukocytes isolated from atopic asthmatic individuals,²⁷⁻³⁰ the current observations raise a novel consideration regarding the pathobiologic mechanisms of asthma. Accordingly, given the important immunologic, proinflammatory, and physiologic responses attributed to FcεRII receptor activation, the current findings support the concept that up-regulated FcεRII expression and action in human asthmatic ASM plays an important role in establishing its asthmatic phenotype.

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HISTOGRAM DISPLAY



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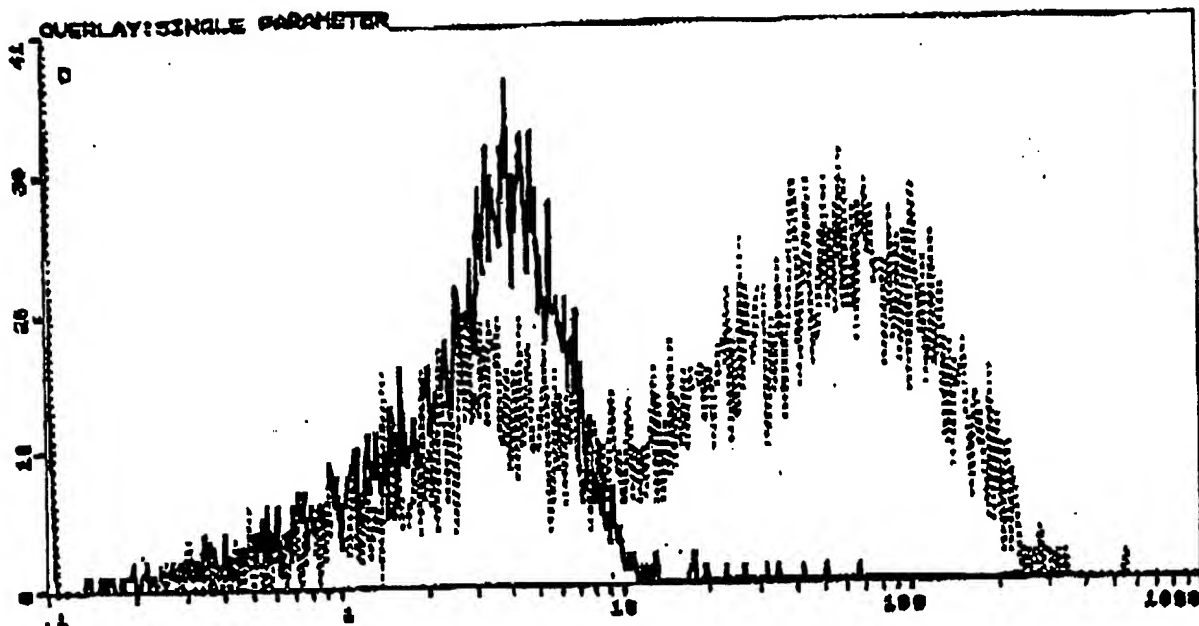
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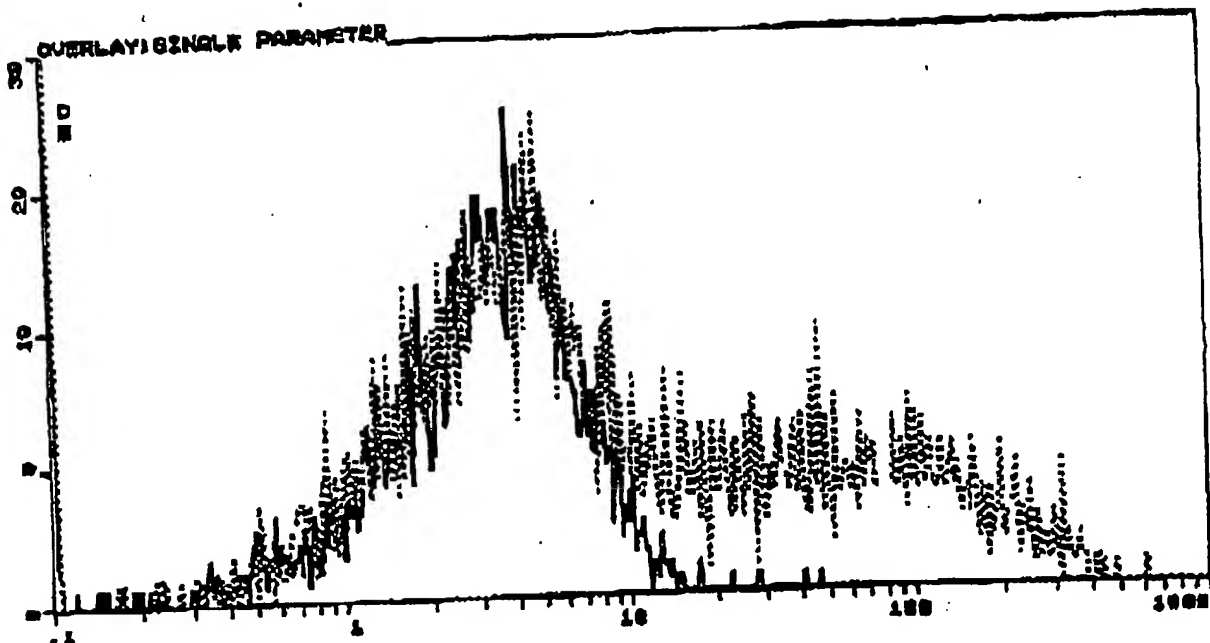


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IgM vs Fc ERII 24 HR ASTHMATIC

Page 1



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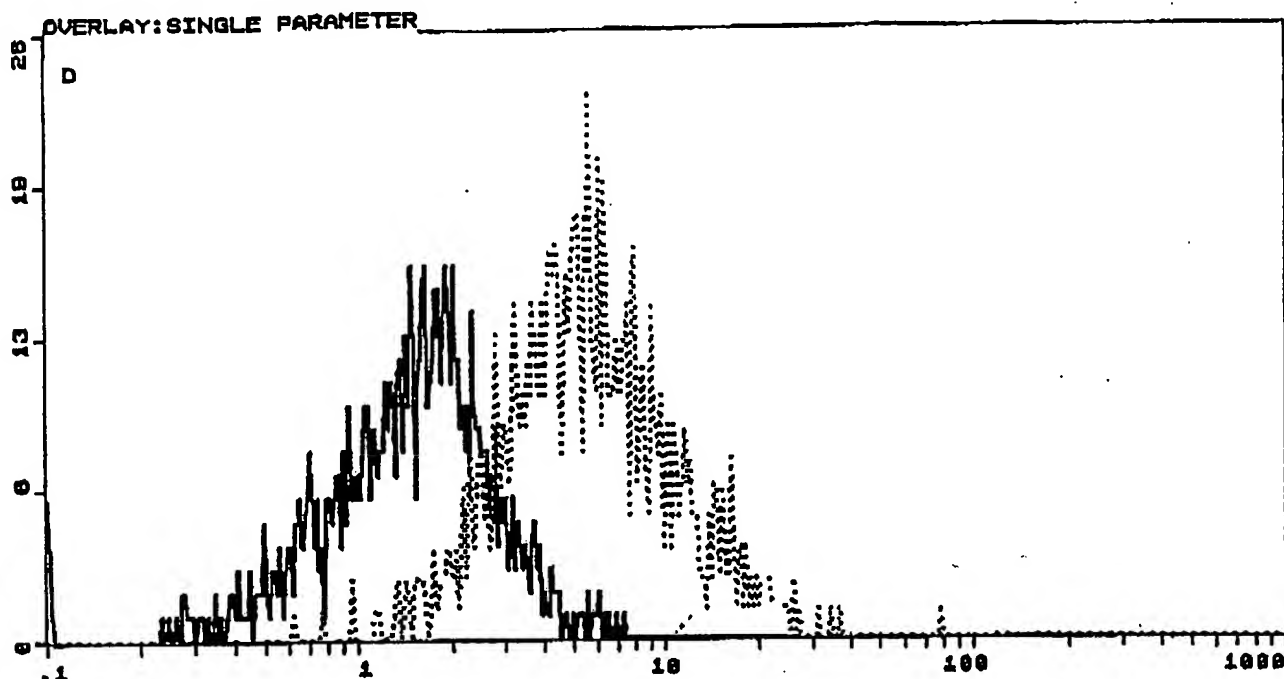
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IGM vs FCE RII 24 HR CONTROL

Page 1

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Antibody T. A. vs F. V. B. M. 24 Hrs. Antibody SERUM

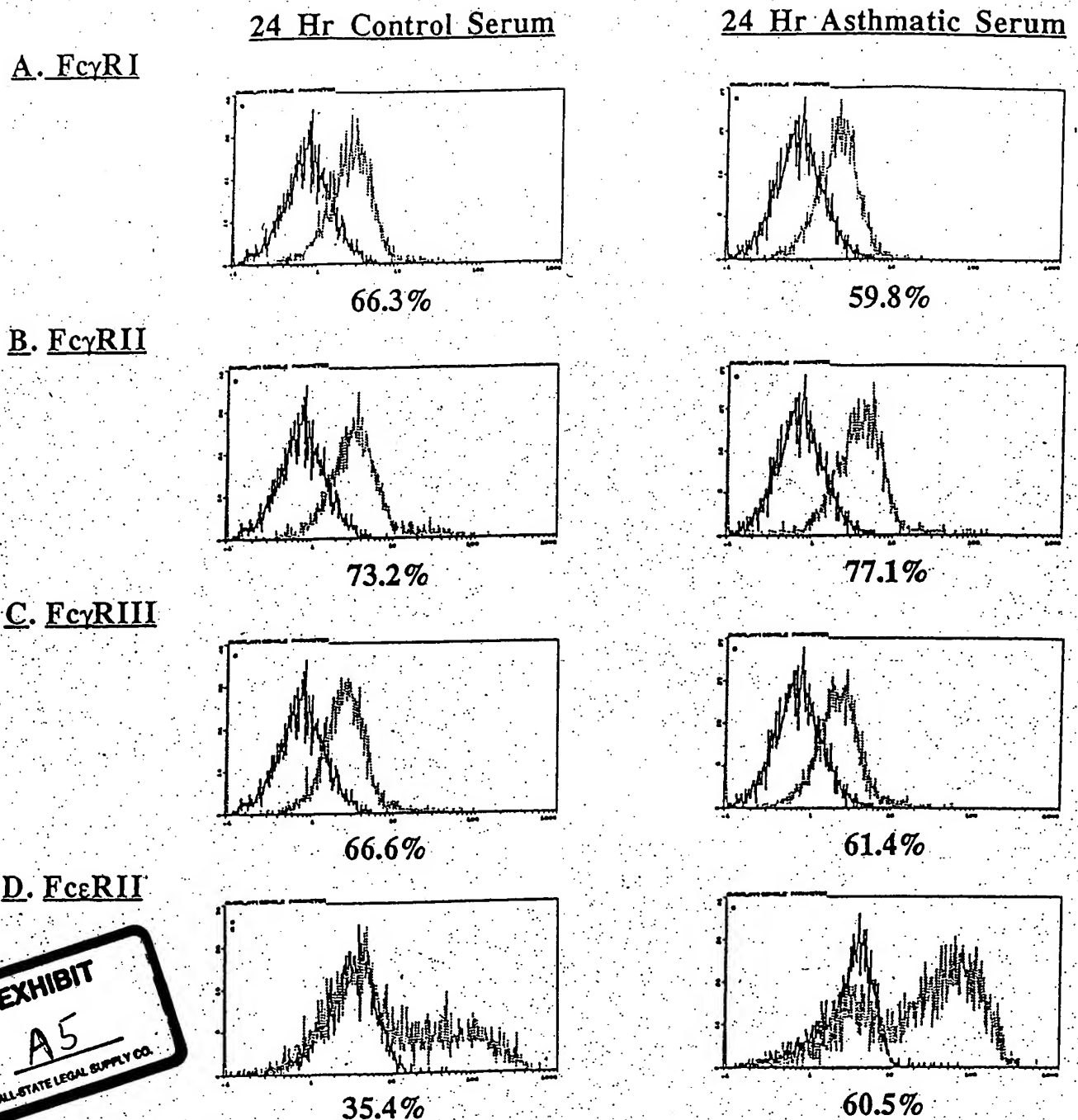
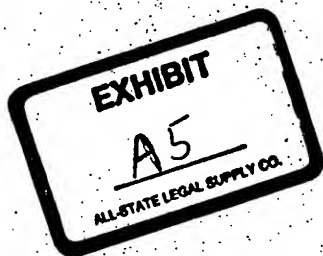


Fig. 14. Flow cytometry of HBSM cells stained with $Fc\gamma RI$, $Fc\gamma RII$, $Fc\gamma RIII$ and $Fc\epsilon RII$ monoclonal antibodies. HBSM cells were treated for 24 hr with 20% control serum (histograms on left), or 20% asthmatic serum (histograms on right) and were stained with FITC-conjugated monoclonal antibodies specific for $Fc\gamma RI$ (A panels), $Fc\gamma RII$ (B panels), $Fc\gamma RIII$ (C panels) and $Fc\epsilon RII$ (D panels) receptors (dotted tracing). The level for non-specific background staining was measured by staining cells with FITC-conjugated isotype control antibodies (solid tracing). The stained HBSM cells were evaluated by flow cytometry using a Coulter EPICS Elite flow cytometer (Coulter EPICS Division, Hialeah, FL) and the fluorescence intensities are expressed as percent positive cells, including mean channel fluorescence.

The above results demonstrate a significant percentage of cells that stain positive for the $Fc\gamma$ receptor subtypes, these data correlate well with the RT-PCR results which indicated mRNA expression for each of these receptor subtypes in HBSM cells. The percent positive cells did not change significantly following the incubation with asthmatic serum. The percent positive cells for the $Fc\epsilon RII$ receptor was nearly two-fold lower than for the $Fc\gamma$ receptors, however, the 24 hr incubation with asthmatic serum resulted in significantly up-regulated expression (171%) of $Fc\epsilon RII$ on the cell surface.



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EXPRESSION OF Fc RECEPTORS BY HUMAN BRONCHIAL SMOOTH MUSCLE CELLS: EFFECT OF HUMAN ATOPIC/ASTHMATIC SERUM. M. Bustillo, H. Hakonarson, M. M. Grunstein, and D.J. Herrick. Division of Pulmonary Medicine, Joseph Stokes, Jr. Research Institute, The Children's Hospital of Philadelphia, University of Pennsylvania School of Medicine, Philadelphia, PA 19104.

This study examined whether human bronchial smooth muscle (HBSM) cells express Fc receptors, and if this expression is modulated by exposure to human atopic/asthmatic serum. We have used RT-PCR and flow cytometry to assay for the expression of Fc receptors on cultured HBSM cells. RT-PCR was used to analyze mRNA expression for the IgG-specific FcγRI, FcγRIIA, C, FcγRIIB1, and FcγRIII receptor subtypes, and for the low affinity IgE FcεRII receptor. cDNA was prepared from total RNA isolated from HBSM cells after 6 h treatment with 20% asthmatic or nonasthmatic serum. PCR results demonstrated that HBSM cells were positive for the FcγRI, FcγRIIA, C, FcγRIII, and FcεRII receptor mRNAs, and negative for the FcγRIIB1 subtype mRNA. The specificity of each PCR product was confirmed by digestion with enzymes at a unique internal site. The other approach demonstrating Fc receptor expression used flow cytometry to quantitate the binding of FcγRI, FcγRII, FcγRIII, and FcεRII FITC-conjugated monoclonal antibodies to the HBSM cell surface. Confluent HBSM cells were treated for 24 h with 20% asthmatic or nonasthmatic serum before staining. These results demonstrated that 60-77% of the cells were positive for the Fcγ receptor subtypes, and that the percentage of Fcγ positive cells did not change with asthmatic vs nonasthmatic serum treatment. The percentage of positive cells expressing the FcεRII receptor was nearly two-fold lower (35.4%) than for the Fcγ receptors; however, the 24 h incubation with asthmatic serum significantly up-regulated the expression to 60.5% of the cells. In conclusion, HBSM cells express both Fcγ and FcεRII receptors, and the latter are up-regulated by exposure to atopic/asthmatic serum.

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ALTERED Fc RECEPTOR EXPRESSION IN ATOPIC/ASTHMATIC SENSITIZED RABBIT AIRWAY SMOOTH MUSCLE. H. Hakonarson, D.J. Herrick, and M.M. Grunstein. Division of Pulmonary Medicine, Joseph Stokes, Jr. Research Institute, The Children's Hospital of Philadelphia, University of Pennsylvania School of Medicine, Philadelphia, PA 19104.

We have previously shown that passive sensitization of rabbit airway smooth muscle (ASM) with human atopic/asthmatic serum produces heightened ASM constrictor responsiveness to acetylcholine (ACh) and attenuated ASM relaxant responsiveness to β -adrenoceptor stimulation. These modulatory effects of atopic/asthmatic serum on the ASM responsiveness were blocked by depleting the serum of immune complexes and were simulated by exogenous administration of immunoglobulin (Ig) G and IgE immune complexes to naive ASM, suggesting that these functional alterations in responsiveness in the sensitized state were mediated by activation of Fc receptors on the ASM. The present study extended these earlier observations to examine whether rabbit ASM cells express Fc receptors and whether the expression of Fc receptors on ASM is modulated by atopic asthmatic serum. Fc receptor expression was determined by: 1) RT-PCR and southern blotting using human-specific Fc γ RI, RII and RIII and Fc ϵ RII primers, and cDNA probes; and 2) flow cytometry using human monoclonal antibodies specific for the individual Fc receptors. cDNA was synthesized from cultured ASM cells following 0, 3, 6 and 24 hr treatment with atopic/asthmatic vs. control serum. Relative to the immortalized 8.1.6 B-cell line, ASM cells expressed mRNA for the Fc γ RIII and the Fc ϵ RII receptors at relatively low levels and their mRNA expression did not change significantly during treatment of the cells with atopic/asthmatic serum. Similarly, ASM cells stained positive for cell surface Fc γ RI, -RII, -RIII receptor expression (40-80%), and their expression of these receptors did not change significantly during treatment with the atopic asthmatic serum. In contrast, expression of the Fc ϵ RII receptor, although present at a low level (15%) in control ASM, was enhanced approximately two fold following treatment with the atopic/asthmatic serum. Collectively, these observations provide new evidence demonstrating that rabbit ASM cells express Fc γ and Fc ϵ RII receptors, and that the cell surface expression of the low-affinity receptor for IgE (Fc ϵ RII) is significantly enhanced by exposure to atopic/asthmatic serum. These findings support the concept that Fc receptors on ASM may mediate the effects of atopic sensitization on ASM responsiveness.

This abstract is funded by: NIH Grant HL-31467; Parker B. Francis Fellowship Award.

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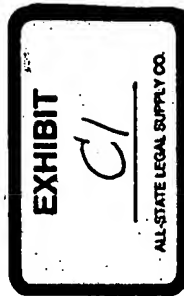
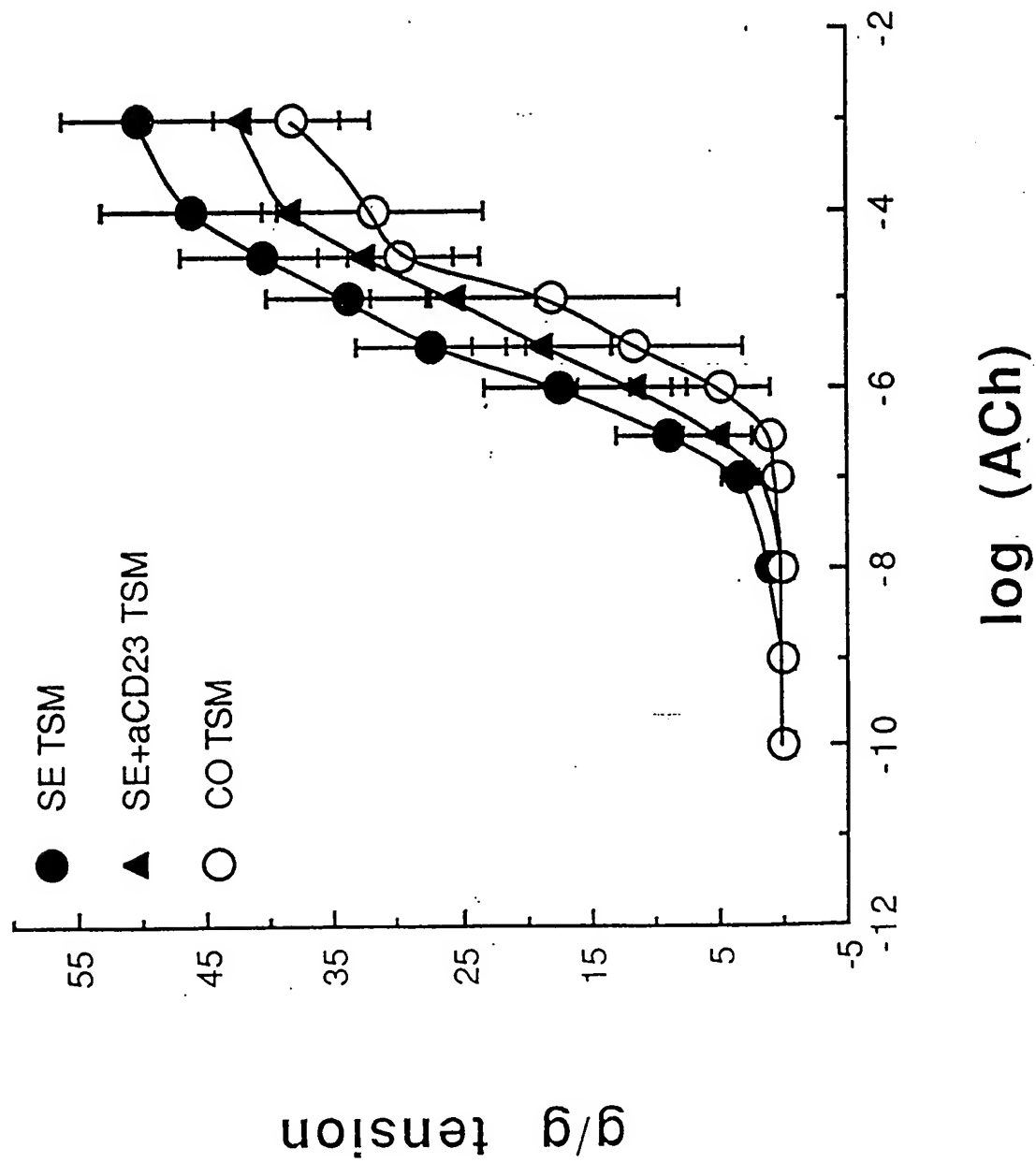
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9		ACH/KCI		0.00	1.70	2.83	8.48	24.32	59.38	82.01	102.46	130.08	152.70				
10																	
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16																	
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19		g/g	0.085	0.00	1.18	3.06	8.24	20.00	30.59	35.29	40.00	44.71	48.24	7.73	(3X10-Z)		
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21		ACH/KCI		0.00	15.22	39.57	106.54	258.73	395.71	458.59	517.46	578.34	624.00				
22																	
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26	CD	%max		0.00	0.00	0.00	0.00	10.87	28.26	45.65	67.39	86.96	100.00				
27		ACH/KCI		0.00	0.00	0.00	0.00	382.50	994.49	1606.49	2321.48	3059.98	3518.97				
28																	
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34																	
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44	CO	%max		0.00	0.00	0.00	0.00	3.13	9.38	25.00	50.00	71.88	100.00				
45		ACH/KCI		0.00	0.00	0.00	0.00	2.46	7.37	19.67	39.33	56.54	78.66				
46																	
47		Reading	9.00	9.00	9.00	9.50	10.00	14.50	22.00	27.00	32.50	35.50	38.00				
48	TSM#8	Ten-Base	0.00	0.00	0.00	0.50	1.00	5.50	13.00	18.00	23.50	26.50	29.00				
49		g/g	0.131	0.00	0.00	0.76	1.53	8.40	19.85	27.48	35.88	40.46	44.27	49.7	CONTROL		
50	CO	%max		0.00	0.00	1.72	3.45	18.97	44.83	62.07	81.03	91.38	100.00				
51		ACH/KCI		0.00	0.00	1.92	9.85	21.15	49.99	69.22	90.37	101.91	111.52				
52																	

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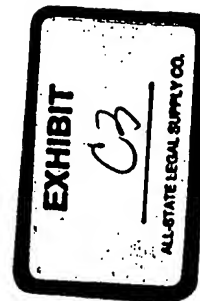
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3	0	0	1.178	3.060	8.240	20.000	30.590	35.290	40.000	44.710	48.240
4	•	•	•	•	•	•	•	•	•	•	•
5	0	0	.949	3.143	8.787	17.377	27.290	33.720	40.247	46.033	50.043
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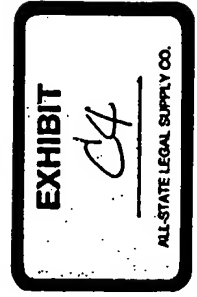
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Robert TBM - Sensitivity $\propto CO_2$ AS : ACO-DRC

	-10	-9	-8	-7	-6.52	-6	-5.52	-5	-4.52	-4	-3
	X ₁	X ₂	X ₃	X ₄	X ₅	X ₆	X ₇	X ₈	X ₉	X ₁₀	X ₁₁
1	0	0	0	0	0	3.290	8.550	13.820	20.390	26.320	30.260
2	0	0	0	1.820	9.090	18.180	27.270	36.360	44.550	52.730	56.360
3	0	0	0	2.020	6.060	13.130	20.200	26.260	32.320	36.360	39.390
4	•	•	•	•	•	•	•	•	•	•	•
5	0	0	0	1.280	5.050	11.533	18.673	25.480	32.420	38.470	42.003
6	0	0	0	.643	2.672	4.372	5.458	6.518	6.975	7.697	7.647

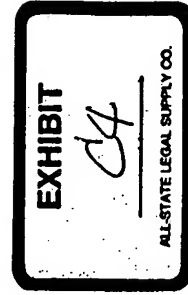
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M
6M



10/1/88 Rabbit TBM - Sensitivity to CO₂ AS : ACO-PRC

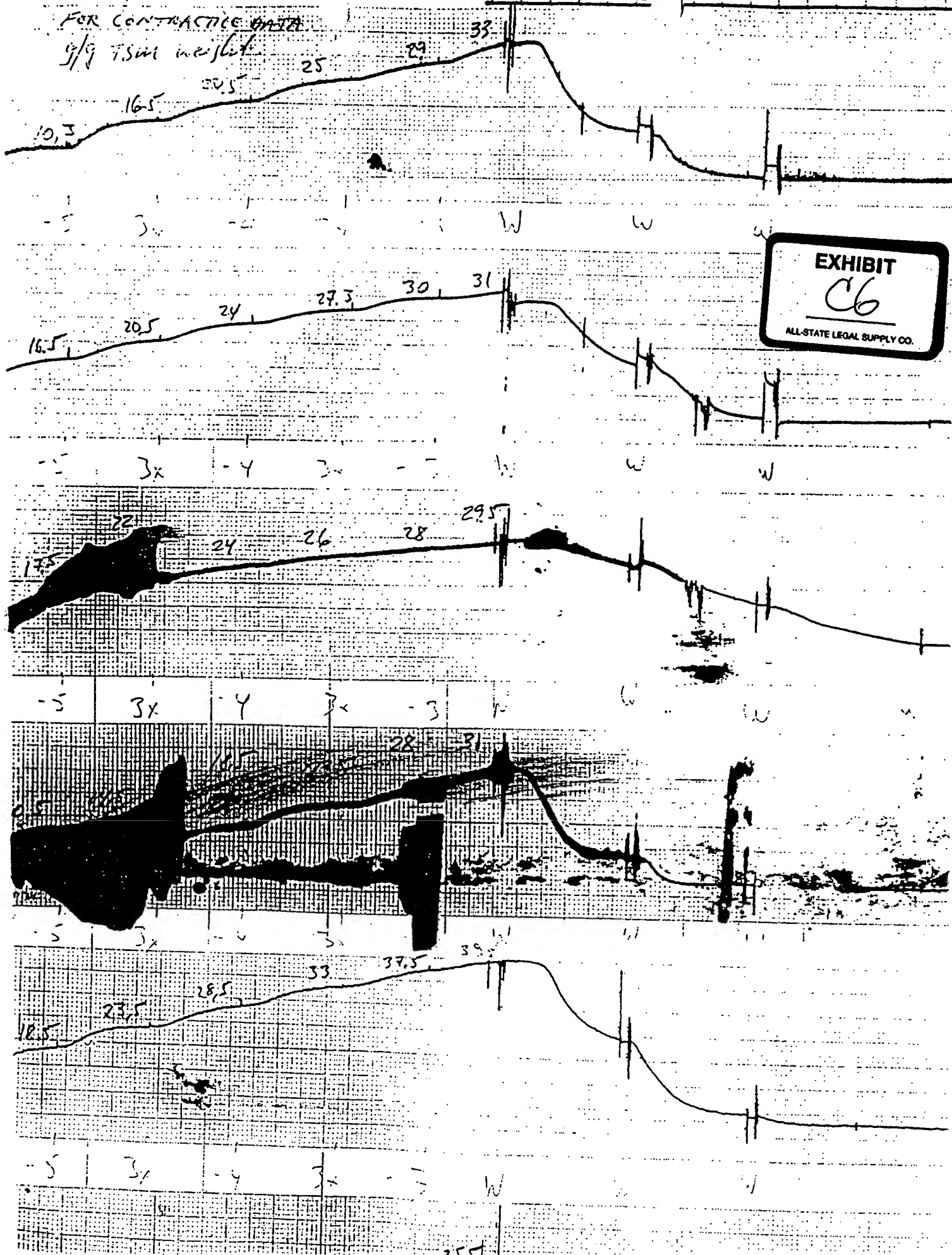
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1	0	0	0	0	0	3.290	8.550	13.820	20.390	26.320	30.260
2	0	0	0	1.820	9.090	18.180	27.270	36.360	44.550	52.730	56.360
3	0	0	0	2.020	6.060	13.130	20.200	26.260	32.320	36.360	39.390
4	•	•	•	•	•	•	•	•	•	•	•
5	0	0	0	1.280	5.050	11.533	18.673	25.480	32.420	38.470	42.003
6	0	0	0	.643	2.672	4.372	5.458	6.518	6.975	7.697	7.647

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EM



FOR CONTRACTOR DATA

9/9 TSMI weight



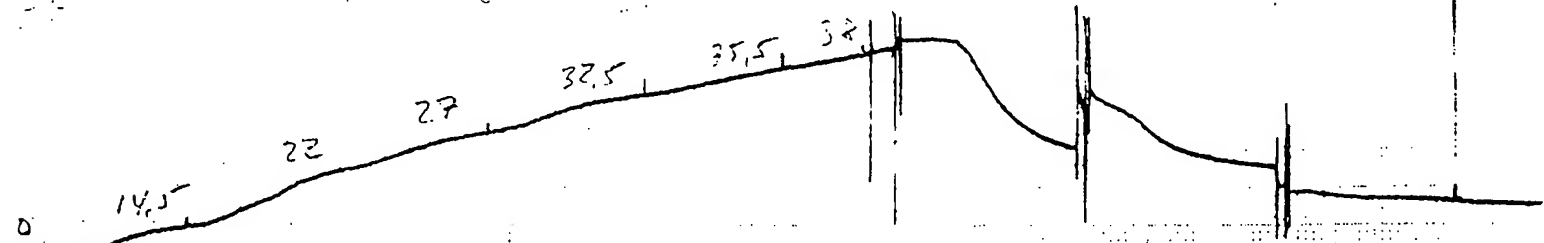
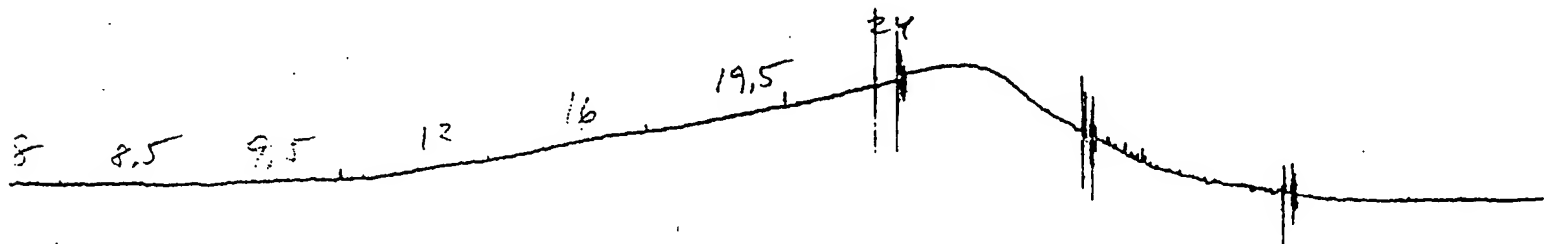
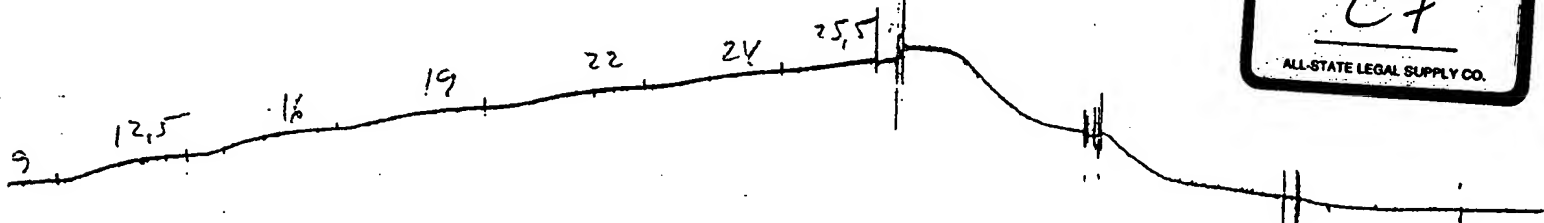
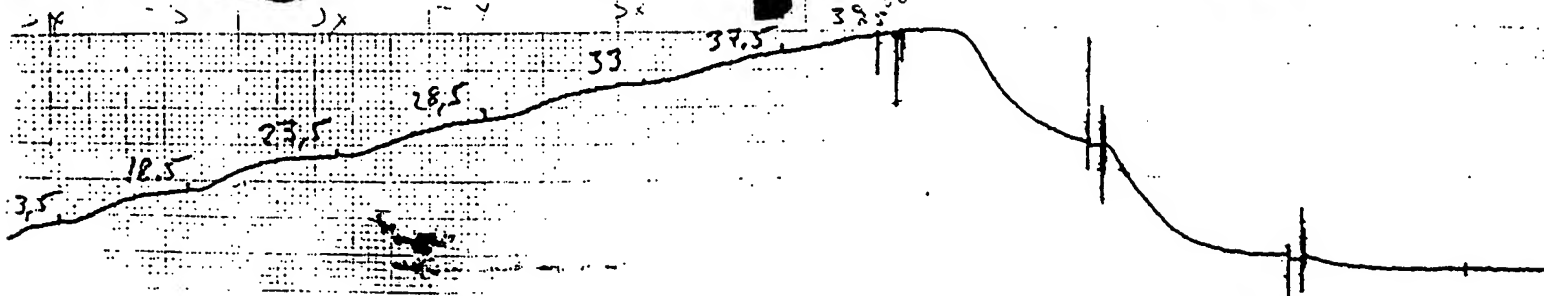
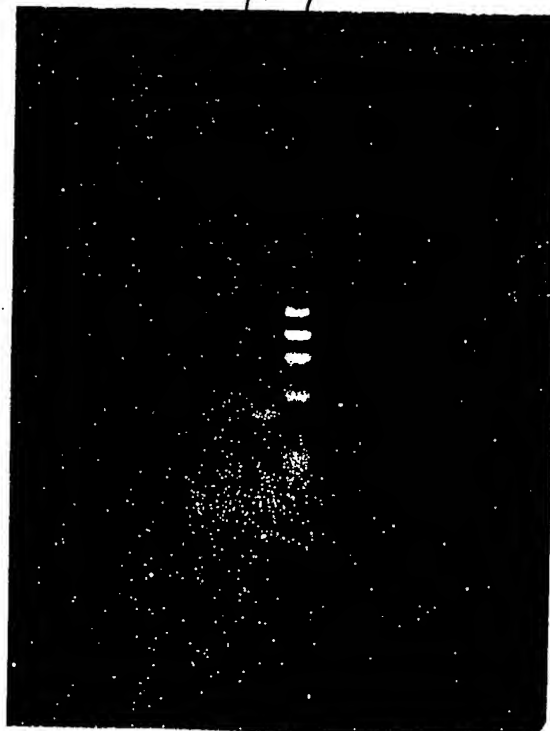


EXHIBIT
C7
ALL-STATE LEGAL SUPPLY CO.

PCR - ON GEL

- ① HBSM CONTROL 6° = Lane
- ② HBSM SENSIT 6° = Lane
- ③ HBSM CONTROL 6° = — : 5
- ④ HBSM SENSIT 6° = — : 6
- ⑤ Φ X-174 marker = — : 7
(1.8 μ l marker & 3.2 μ l 0.1XTAE)
1ul dye

CO// AS//



ORDER ON 30 ml, 1.2% AGAROSE
IN THE MINI-GEL

- (1) IGFBP-2 PCR Q FRAG (H₂O)
- (2) " " " " (TRIS)
- (3) IGFBP-3 " " " (H₂O)
- (4) " " " " (TRIS)

2.5 µl DNA

- (5) ΦX174 c Hae-III 2 µl DNA

- (6) GHR CONTROL RTSM } IL-1P PRIMERS

- (7) " ASTHM. " }

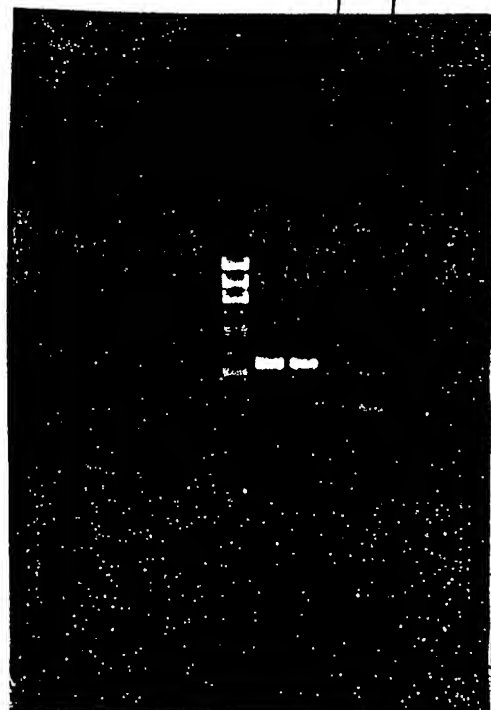
5 µl DNA

- (8) " CONTROL " }

FCER1 PRIMERS

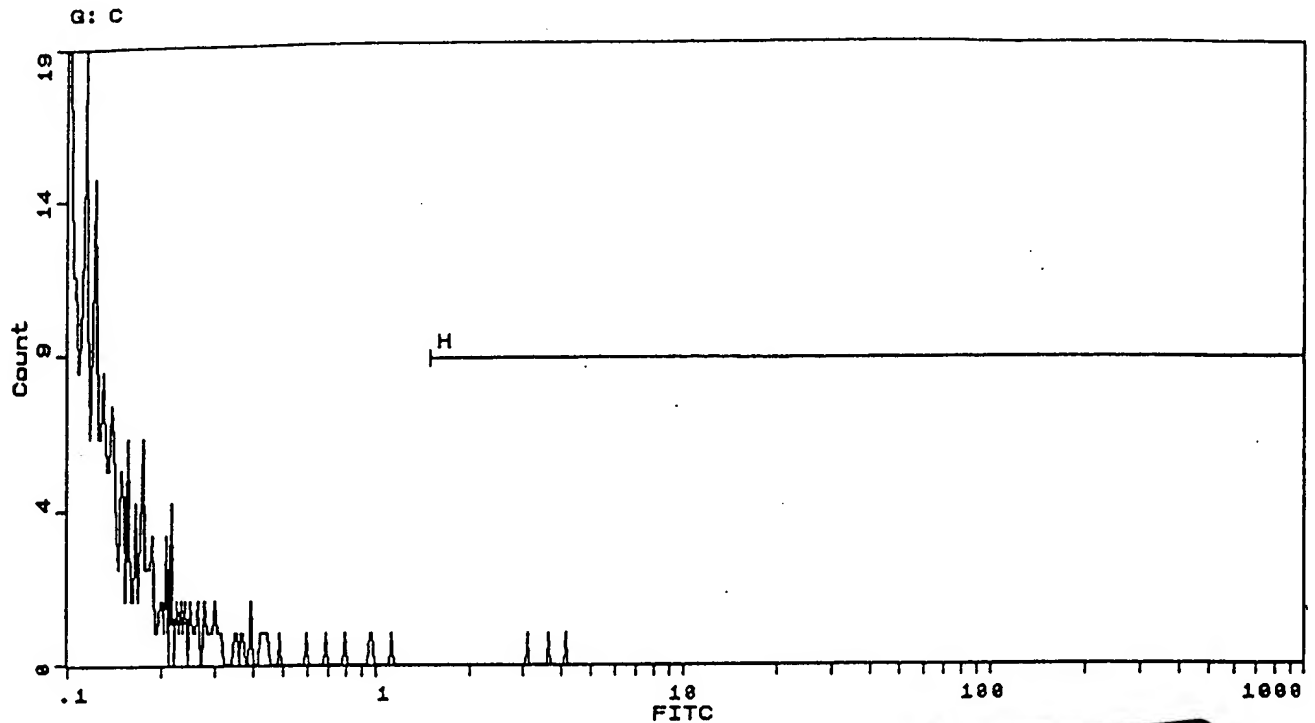
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CO AS



SAMPLE NAME : 1A
 SAMPLE NUMBER:

SAMPLE DATE:
 SAMPLE TIME: 11:53:57



----- SAMPLE INFO -----

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 DATA RATE : 113
 INSTRUMENT : Elite
 SAMPLE NAME : 1A
 SAMPLE NUMBER:
 COMMENTS :

HIST COUNT : 2,000

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 PROT FILE : HB000049.PRO
 SAMPLE DATE:
 SAMPLE TIME: 11:53:57

----- STATISTICS -----

SINGLE PARAMETER STATISTICS

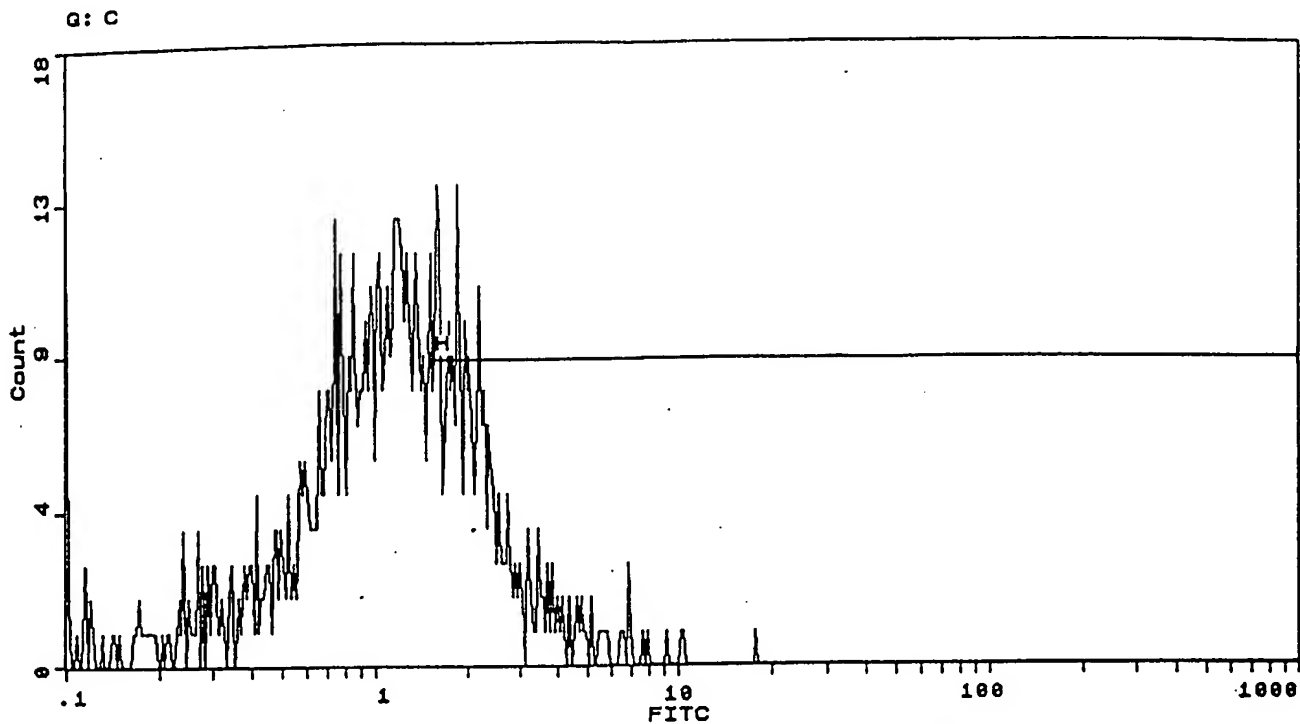
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H Low Channel = 1.5, High Channel = 1024 H

Lab instrument control, scripted

SAMPLE NAME : 1A
 SAMPLE NUMBER:

SAMPLE DATE: .
 SAMPLE TIME: 11:51:59



EXHIBIT

E2

ALL-STATE LEGAL SUPPLY CO.

----- SAMPLE INFO -----

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 INSTRUMENT : Elite
 SAMPLE NAME : 1A
 SAMPLE NUMBER:
 COMMENTS :

HIST COUNT : 2,000

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 PROT FILE : HB000049.PRO
 SAMPLE DATE:
 SAMPLE TIME: 11:51:59

----- STATISTICS -----

SINGLE PARAMETER STATISTICS

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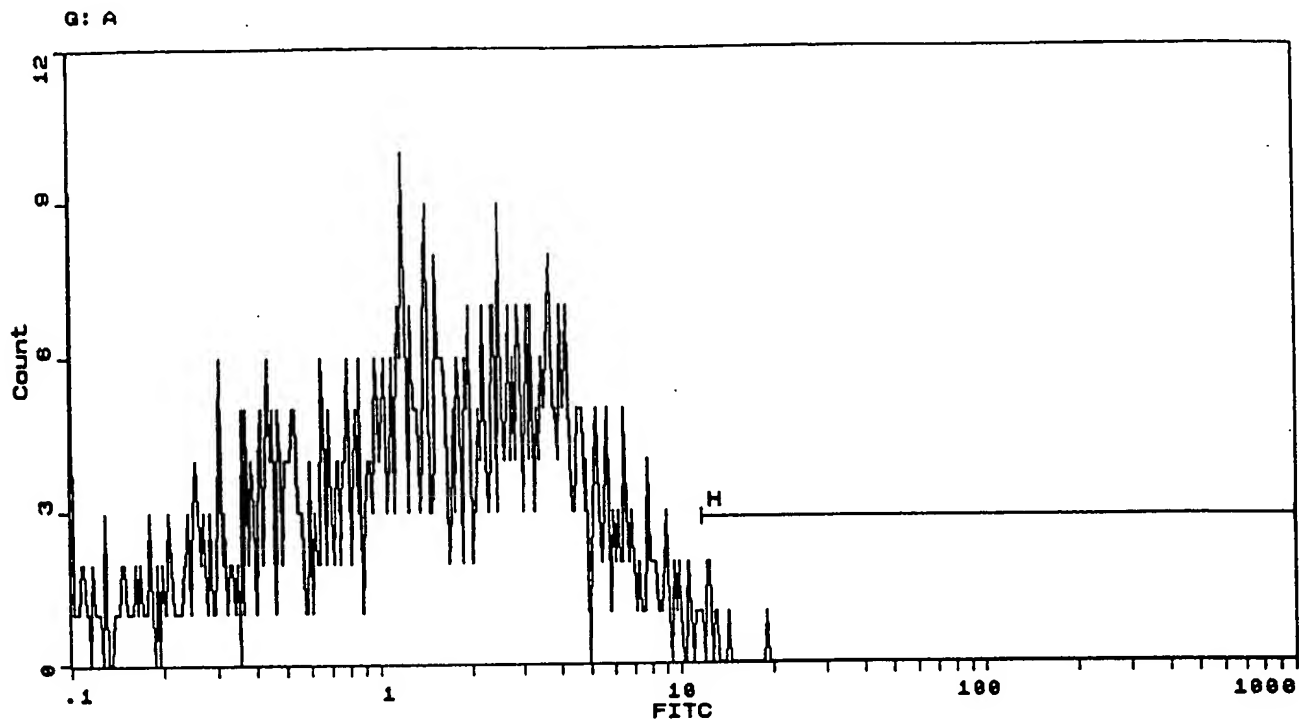
H Low Channel = 1.5, High Channel = 1024 H

CDZ5 - Sealed -

GATES

SAMPLE NAME : IGG1 FITC CT
 SAMPLE NUMBER:

SAMPLE DATE: J
 SAMPLE TIME: 14:39:29



EXHIBIT

E3

ALL-STATE LEGAL SUPPLY CO.

----- SAMPLE INFO -----

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 INSTRUMENT : Elite
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 COMMENTS :

HIST COUNT : 2,000

LIST FILE :
 PROT FILE : HB000049.PRO
 SAMPLE DATE:
 SAMPLE TIME:

----- STATISTICS -----

SINGLE PARAMETER STATISTICS

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H	1.8	36	12	4	20.6	19.3	93.6	0.382	12	1024

H Low Channel = 12, High Channel = 1024 H

Indirigo.

T, MA AT

AT

Human Lung & TSM

probed w/ FCE

Exposed 5 1/2 hrs.



C A

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LUNG

ASUM

PROVISIONAL APPLICATION
FILING RECEIPT



UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office
ASSISTANT SECRETARY AND COMMISSIONER
OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

APPLICATION NUMBER	FILING DATE	FIL FEE REC'D	ATTORNEY DOCKET NO.	DRWGS
60/077,398	03/10/98	\$75.00	7600-22US	10

KATHRYN DOYLE LEARY
PANITCH SCHWARZE JACOBS & NADEL
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2005 MARKET STREET - 22ND FLOOR
PHILADELPHIA PA 19103-7086

Receipt is acknowledged of this Provisional Application. This Provisional Application will not be examined for patentability. Be sure to provide the PROVISIONAL APPLICATION NUMBER, FILING DATE, NAME OF APPLICANT, and TITLE OF INVENTION when inquiring about this application. Fees transmitted by check or draft are subject to collection. Please verify the accuracy of the data presented on this receipt. If an error is noted on this Filing Receipt, please write to Box Provisional Application within 10 days of receipt. Please provide a copy of the Provisional Application Filing Receipt with the changes noted thereon. This Provisional Application will automatically be abandoned twelve (12) months after its filing date and will not be subject to revival to restore it to pending status beyond a date which is after twelve (12) months from its filing date.

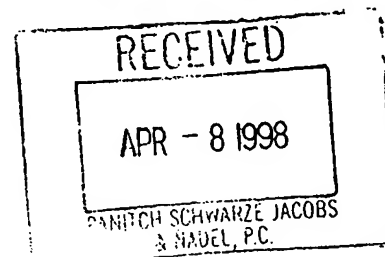
Applicant(s) MICHAEL M. GRUNSTEIN, MERION STATION, PA; H. HAKONARSON,
WAYNE, PA.

FOREIGN FILING LICENSE GRANTED 04/04/98

* SMALL ENTITY *

TITLE

COMPOSITIONS AND METHODS FOR TREATMENT OF ASTHMA



Handwritten signature

KD/LCH EL 004273565US

ATTORNEY DOCKET # 7600-2245 TODAY'S MAILING DATE 3/10/98

PAT/TM/S. N. / REG/OPP/CANC./# _____

CERT. of MAIL/CERT. of SERV/EXPRESS MAIL

OF:

Michael M. Brunstein et al.

FOR:

Compositions and Methods for treatment of asthma

RECEIPT IS ACKNOWLEDGED FOR THE FOLLOWING:

TRADEMARK APPLICATION _____

Specimens

RENEWAL/AFF 8/AFF 8 & 15

PAT APP (NEW/DIV/CIP/FWC/RE)

38 Dec & Power of Atty

Pages Specifications

Preliminary Amendment

Claims

Sheets Drawings

Type _____

COVER SHEET/LETTER/ORDER

AMENDMENT/REQ. RECONS

ASSIGN/CHG NAME/MERGER/SEC. INT.

VER STMT/SMALL ENTITY

NOTICE OF APPEAL/BRIEF _____ COPIES

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PRIORITY DOC. _____

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Fee authorization charge Deposit Acct. #16-0235 \$ 75.00

OTHER (PAPER TITLE) _____

EL004273565US

PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53 (c).

Attorney Docket Number: 7600-22US			Type a plus sign (+) inside this box: [+]
INVENTOR(s) APPLICANT(s)			
LAST NAME	FIRST NAME	MIDDLE INITIAL	RESIDENCE (CITY AND EITHER STATE OR FOREIGN COUNTRY)
Grunstein Hakonarson	Michael H.	M.	Merion Station, PA 19066 Wayne, PA 19087
TITLE OF INVENTION (280 characters maximum)			
Compositions And Methods For Treatment of Asthma			
CORRESPONDENCE ADDRESS			
KATHRYN DOYLE LEARY, Ph.D., J.D. PANITCH SCHWARZE JACOBS & NADEL, P.C. One Commerce Square - 2005 Market Street - 22nd Floor Philadelphia, PA USA 19103-7086		Telephone: (215) 567-2020 Facsimile: (215) 567-2991 E-Mail: kdl@psjn.com Direct Dial: (215) 965-1284	
ENCLOSED APPLICATION PARTS (check all that apply)			
<input checked="" type="checkbox"/> Specification - Number of Pages: 58		<input checked="" type="checkbox"/> Small Entity Statement	
<input type="checkbox"/> Drawing(s) - Number of Sheets:		<input type="checkbox"/> Other (specify):	
METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT (check one)			
<input type="checkbox"/> A check or money order is enclosed to cover the Provisional filing fee.			
<input checked="" type="checkbox"/> The Commissioner is hereby authorized to charge filing fees or credit Deposit Account Number: 16-0235 (Attach duplicate copy of this page if paying by deposit account).			
<input checked="" type="checkbox"/> PROVISIONAL FILING FEE AMOUNT: \$75.			
The invention was made by an agency of the United States Government or under a contract with an agency of the U.S. Government.			
<input type="checkbox"/> No, or <input checked="" type="checkbox"/> Yes, the name of the U.S. Government agency and the Government contract number are: National Heart, Lung and Blood Institute, Grant No. HL-31467			
March 10, 1998 (Date)		Respectfully submitted, By:  KATHRYN DOYLE LEARY, Ph.D., J.D. Registration No. 36,317	
<input type="checkbox"/> Additional inventors are being named on separately numbered sheets attached hereto.			

PROVISIONAL APPLICATION FILING ONLY

MODIFIED

COMPOSITIONS AND METHODS FOR TREATMENT OF ASTHMA

GOVERNMENT SUPPORT

This invention was made in part using funds obtained from the U.S.
5 Government (National Heart, Lung and Blood Institute Grant No. HL-31467) and the
U.S. Government may therefore have certain rights in the invention.

FIELD OF THE INVENTION

The field of the invention is asthma therapy.

BACKGROUND OF THE INVENTION

10 Bronchial asthma in mammals is characterized by inflammation of the
airways, exaggerated airway reactivity to bronchoconstrictor agonists, and attenuated
beta-adrenoceptor-mediated airway relaxation (Bai, 1990 Am. Rev. Respir. Dis.
141:552-557; Goldie et al., 1986, Br. J. Clin. Pharmacol. 22:669-676; McFadden et
al., 1994, Am. J. Respir. Crit. Care Med. 150:523-526). In humans with atopic
15 asthma, mast cell activation has been implicated in mediating the immediate
bronchoconstrictor response which acutely follows antigen inhalation. This response
is a process which involves IgE-mediated activation of the high affinity IgE receptor
(FcεRI), leading to cellular degranulation and the release of various mast cell-derived
mediators including histamine, eicosanoids, and specific cytokines (Metzger, 1992,
20 Immunol. Rev. 125:37-48; Beaven et al., 1993, Immunol. Today 14:222-226; Galli,
1993, N. Engl. J. Med. 328:257-265). The identification of Fc receptors on other cell
types in the lung (e.g., mononuclear cells, eosinophils, and dendritic cells) suggests
that, apart from mast cells per se, these other cell types may also serve to propagate the
pro-inflammatory allergic pulmonary response, most likely via the orchestrated
25 extended release of various cytokines (Walker et al., 1992, Am. Rev. Respir. Dis.

146:109-115; Watson et al., 1993, *Am. J. Respir. Cell Mol. Biol.* 8:365-369; Capron et al., 1984, *J. Immunol.* 132:462-468; Beasley et al., 1989, *Am. Rev. Respir. Dis.* 139:806-817; Litchfield et al., 1992, *J. Asthma* 29:181-191; Barnes et al., 1988, *Pharmacol. Rev.* 40:49-84; Borish et al., 1991, *J. Immunol.* 146:63-67. It is believed
5 that immune complex/Fc receptor interactions expressed by these cells, i.e., mononuclear cells, eosinophils, and dendritic cells, potentially underlie the progression of the airway inflammatory and bronchoconstrictor responses in asthma, wherein the immediate bronchoconstriction accompanying antigen exposure is followed by the development of the late phase asthmatic response involving various
10 proinflammatory cells. Indeed, recent studies have demonstrated that expression of the inducible form of the low affinity IgE receptor (FcεRII or CD23) is upregulated on monocytes and alveolar macrophages (Williams et al., 1992, *J. Immunol.* 149:2823-2829), as well as on circulating B lymphocytes (Gagro et al., 1993, *Int. Arch. Allergy Immunol.* 101:203-208; Rabatic et al., 1993, *Exp.*
15 *Immunol.* 94:337-340) isolated from atopic asthmatic subjects. Similarly, exposure of asthmatic subjects to allergen and treatment of isolated monocytes with specific cytokines have been shown to up-regulate FcεRII expression on mononuclear phagocytes (Williams et al., 1992, *J. Immunol.* 149:2823-2829; Joseph et al., 1983, *J. Clin. Invest.* 71:221-230). These findings suggest that altered Fc receptor expression
20 and action in some cell types may contribute to the overall pro-inflammatory asthmatic response. While it is known that exposure of isolated rabbit and human airway smooth muscle (ASM) to atopic asthmatic serum induces the autocrine release and action of specific cytokines (notably IL-1β) by the sensitized ASM cells (Hakonarson et al., 1997, *J. Clin. Invest.* 99:117-124), the mechanism by which this
25 sensitization is mediated has not been disclosed.

Current treatment options for asthma include medications that control the airway inflammatory component of the disease, e.g., primarily corticosteroids, sodium cromolyn, methylxanthines, leukotriene modifiers) and rapid relief medications that counteract bronchospasm, e.g., primarily beta-adrenergic agents.

There are several disadvantages to using these medications as follows: There is a potential lack of effective sustained action; there are side effects associated with prolonged use of these medications, particularly in the case of corticosteroids and beta-adrenergic agents; there is a progressive loss of sensitivity to these treatments after prolonged use; there is limited efficacy of any of these agents in severe cases of asthma; these agents are non-selective, i.e., they do not specifically target the lung, therefore, side-effects affecting other organs are a potential risk. Furthermore, there are data which document an increased risk of dying from bronchial asthma following prolonged treatment of asthma using long-acting beta-adrenergic agents such as fenoterol (Pearce et al., 1990, Thorax 45:170-175; Spitzer et al., 1992, New Engl. J. Med. 326:560-561).

Approximately fifteen million individuals in the U.S. have asthma and the disease is the cause of more than five thousand deaths annually in the U.S. In children, asthma represents the most prevalent chronic disease, requiring the most frequent use of emergency room visits and hospitalizations. The overall annual cost for asthma care in the U.S. is estimated to be about nine billion dollars. Asthma is the most common cause of school and work absenteeism in the U.S.

There is thus a long felt need for additional and more specific and effective compositions and methods for treatment of asthma which additional compositions and methods overcome the deficiencies of the prior art compositions and methods.

SUMMARY OF THE INVENTION

The invention relates to a method of preventing induction of an asthmatic state in a human patient comprising administering to the human an anti-FcεRII receptor protein ligand suspended in a pharmaceutically acceptable carrier in an amount sufficient to inhibit binding of IgE to an anti-FcεRII receptor protein thereby preventing induction of the asthmatic state in the human. Preferably, the pharmaceutically acceptable carrier is physiological saline.

In one aspect, the ligand is selected from the group consisting of an isolated protein, an isolated polypeptide and a non-peptide.

In a preferred embodiment, the ligand is selected from the group consisting of a polyclonal antibody, a monoclonal antibody, a synthetic antibody, a chimeric antibody and a humanized antibody. Preferably, the anti-FcεRII receptor protein ligand is an anti-FcεRII receptor protein antibody.

In another aspect, the ligand is administered to the human in the form of an isolated DNA encoding and capable of expressing the ligand. The DNA may be formulated in a viral or a non-viral vector. When the DNA is formulated in a viral vector, the viral vector is selected from the group consisting of a recombinant vaccinia virus, a recombinant adenovirus, a recombinant retrovirus, a recombinant adeno-associated virus and a recombinant avian pox virus. When the DNA is formulated in a non-viral vector, the non-viral vector is selected from the group consisting of a liposome and a polyamine conjugated DNA.

In yet another aspect, the anti-FcεRII receptor protein ligand is administered to the human in an amount between about 1 ng/kg and about 100 mg/kg of patient body weight.

The invention also relates to a method of treating asthma in a human patient comprising administering to the human an anti-FcεRII receptor protein ligand suspended in a pharmaceutically acceptable carrier in an amount sufficient to inhibit binding of IgE to an anti-FcεRII receptor protein thereby treating asthma in the human. Preferably, the pharmaceutically acceptable carrier is physiological saline.

In one aspect, the ligand is selected from the group consisting of an isolated protein, an isolated polypeptide and a non-peptide.

In a preferred embodiment, the ligand is selected from the group consisting of a polyclonal antibody, a monoclonal antibody, a synthetic antibody, a chimeric antibody and a humanized antibody. Preferably, the anti-FcεRII receptor protein ligand is an anti-FcεRII receptor protein antibody.

In another aspect, the ligand is administered to the human in the form of an isolated DNA encoding and capable of expressing the ligand. The DNA may be formulated in a viral or a non-viral vector. When the DNA is formulated in a viral vector, the viral vector is selected from the group consisting of a recombinant vaccinia virus, a recombinant adenovirus, a recombinant retrovirus, a recombinant adeno-associated virus and a recombinant avian pox virus. When the DNA is formulated in a non-viral vector, the non-viral vector is selected from the group consisting of a liposome and a polyamine conjugated DNA.

In yet another aspect, the anti-FcεRII receptor protein ligand is administered to the human in an amount between about 1 ng/kg and about 100 mg/kg of patient body weight.

Also included in the invention is a method of treating a human having asthma comprising administering to the human a pharmaceutically effective amount of an isolated nucleic acid encoding an anti-FcεRII receptor protein ligand, wherein the nucleic acid expresses the ligand in vivo in an amount sufficient to treat the asthma.

In addition, the invention includes a method of identifying an anti-FcεRII receptor protein ligand comprising providing a mixture comprising IgE and a population of cells which express an FcεRII receptor protein, incubating the mixture in the presence or absence of a test ligand, and measuring the level of IgE bound to the FcεRII receptor protein, wherein a lower level of IgE bound to the cells in the presence of the test compound compared with the level of binding of IgE to the cells in the absence of the test compound is an indication that the test compound is an anti-FcεRII receptor protein ligand.

In a preferred embodiment of this aspect of the invention, the cells are airway smooth muscle cells.

The invention also includes a ligand useful for inhibiting binding of IgE to an FcεRII receptor protein identified by a method comprising providing a mixture comprising IgE and a population of cells which express an FcεRII receptor protein, incubating the mixture in the presence or absence of a test ligand, and measuring the

level of IgE bound to the FcεRII receptor protein, wherein a lower level of IgE bound to the cells in the presence of the test compound compared with the level of binding of IgE to the cells in the absence of the test compound is an indication that the test compound is an anti-FcεRII receptor protein ligand.

- 5 In addition, the invention includes a method of inhibiting binding of IgE to an FcεRII receptor protein expressed on a cell comprising administering to the cell an anti-FcεRII receptor protein ligand.

BRIEF DESCRIPTION OF THE DRAWINGS

- 10 **Figure 1A** is a graph depicting a comparison of contractile dose-response relationships to acetylcholine (ACh) in paired control serum-treated (open circles) and atopic asthmatic serum-treated tracheal smooth muscle (TSM) segments in the absence (closed circles) and presence (open squares) of anti-CD23 monoclonal antibody (MAb).

- 15 **Figure 1B** is a graph depicting a comparison of contractile dose-response relationships to augmented constrictor responses (ACR) in control serum-treated (open circles) and atopic asthmatic serum-treated TSM in the absence (closed circles) and presence (open squares) of Staphylococcus protein A (SpA). Data represent mean \pm SE values from 6 paired tissue samples.

- 20 **Figure 2A** is a graph depicting a comparison of relaxation dose-relationships to isoproterenol in paired control serum-treated (open circles) and atopic asthmatic serum-treated TSM segments half-maximally contracted with their respective ED₅₀ doses of ACh in the absence (filled circles) and presence (open squares) of anti-CD23 MAb.

- 25 **Figure 2B** is a graph depicting a comparison of relaxation responses to isoproterenol in control (open circles) and atopic asthmatic serum-treated TSM in the absence (filled circles) and presence (open squares) of SpA. Data are mean \pm SE values from 8 paired tissue samples.

Figure 3 is a graph depicting a comparison of airway constrictor responses to ACh in isolated paired TSM segments in the absence (open circles) and presence (filled circles) of IgE immune complexes (see Methods). Data represent mean \pm SE values from 6 paired tissue samples.

5 **Figure 4** is a graph depicting a comparison of airway relaxation responses to isoproterenol in paired TSM segments in the absence (open symbols) and presence (filled symbols) of IgE immune complexes (see Methods). All TSM segments were initially half-maximally contracted with their respective ED₅₀ doses of ACh prior to isoproterenol administration. Data represent mean \pm SE values from 8
10 paired tissue samples.

Figure 5A is an image of a gel depicting expression of Fc γ RIII receptor mRNA using reverse transcriptase polymerase chain reaction (RT-PCR) in rabbit ASM cells following 0, 6 and 24 hours of treatment with 10% control serum and 10% atopic asthmatic sensitizing serum. Expression of α -actin was used to control for loading.
15 cDNA obtained from activated U937 cells (i.e., for Fc γ RIII) was used as a positive control. The blots were probed with human specific Fc γ RIII ³²P-labeled cDNA probes.

Figure 5B is an image of a gel depicting expression of Fc ϵ RII receptor mRNA using RT-PCR in rabbit ASM cells following 0, 6 and 24 hours of treatment with 10% control serum and 10% atopic asthmatic sensitizing serum. Expression of α -
20 actin was used to control for loading. cDNA obtained from the immortalized human B-cell line 8.1.6 (i.e., for Fc ϵ RII) was used as a positive control. The blots were probed with human specific Fc ϵ RII ³²P-labeled cDNA probes.

Figure 6A is an image of a Southern blot probed with full length Fc γ RIII and a 157-bp RPL7 human cDNA probe. Paired human airway smooth
25 muscle samples were incubated with control (CO) or atopic asthmatic (SE) serum for 24 hours. cDNA was transcribed from total RNA primed with oligo(dT). PCR products were amplified using a human-specific Fc γ RIII and RPL7 primers, run on 1.2% agarose gels, transferred to a Zeta-probe membrane and then probed.

Figure 6B is an image of a Southern blot probed with full length FcεRII and a 157-bp RPL7 human cDNA probe. Paired human airway smooth muscle samples were incubated with control (CO) or atopic asthmatic (SE) serum for 24 hours. cDNA was transcribed from total RNA primed with oligo(dT). PCR products were amplified using human-specific FcεRII and RPL7 primers, run on 1.2% agarose gels, transferred to a Zeta-probe membrane and then probed.

Figure 7 is an image of a gel depicting a expression of FcεRII mRNA as assessed by RT-PCR in human bronchial smooth muscle tissue following 24 hours of treatment with control (CO) or atopic asthmatic serum in the absence (SE) and presence (SE+) of anti-CD23 MAb. The products of the RT-PCR reactions using 2.5 μg of total RNA and human-specific primers for the FcεRII receptor mRNA are shown. Expression of RPL7 was used to control for gel loading. The blot was probed with the above ³²P-labeled human-specific FcεRII and RPL7 cDNA probes.

Figure 8A is a gel depicting of expression of FcεRII as assessed by RT-PCR in rabbit ASM cells following 0 and 24 hr treatment with media alone (control) or with IgE immune complexes for 0, 6, 12 and 24 hours. The products of RT-PCR reactions using 2.5 μg of total RNA and human-specific primers for the FcεRII receptor mRNA are shown. mRNA expression of α-actin is also shown for comparison.

Figure 8B is a graph depicting the corresponding time-dependent changes in the FcεRII/α-actin ratio, obtained from the data presented in Figure 8A, expressed as fold increase above baseline (i.e., time 0) in control (open bars) and IgE immune complex-treated cells (filled bars).

Figure 9 is a series of graphs comprising panels A-D, depicting a flow cytometric analysis of FcγRIII and FcεRII surface expression in rabbit ASM cells. Cells treated for 24 hours with either 10% control serum or 10% atopic asthmatic serum were stained with fluorescein isothiocyanate (FITC)-conjugated human monoclonal antibodies specific for the low-affinity FcγRIII (Panel A) and FcεRII (CD23) (Panel B) receptors. Activated B-cells (8.1.6) were used as a positive control

for the CD23 receptor. The level of non-specific background staining was measured in both the control and atopic asthmatic serum-treated cells by staining with FITC-conjugated isotype control antibodies.

5 **Figure 10** is a series of images, comprising Panels A and B, depicting immunofluorescence staining for CD23 surface receptor protein in control (CO) serum-treated (left panels) and atopic asthmatic serum (SE) sensitized (right panels) rabbit
10 ASM cells (A; magnification x 100) and TSM tissue (B; magnification x 50). F(ab')₂-FITC conjugated fragments were used to detect the primary monoclonal anti-CD23 antibody. Isotype negative control antibody was used to control for nonspecific staining (upper panels: A and B).

DETAILED DESCRIPTION

The invention relates to the discovery of the expression of an Fc receptor protein on airway smooth muscle cells, which expression plays a significant role in the development of the asthmatic state in an individual having asthma. While
15 expression of the receptor of the invention on other cell types is known, expression of the receptor on airway smooth muscle cells has been heretofore unknown.

The receptor of the invention is a receptor for IgE termed FcεRII or CD23. This receptor is a low affinity receptor for IgE which has been observed to be expressed in an inducible form on monocytes and lung alveolar macrophages (Williams
20 et al., 1992, J. Immunol. 149:2823-2829; Joseph et al., 1983, J. Clin. Invest. 71:221-230), as well as on circulating B lymphocytes (Gagro et al., 1993, Int. Arch. Allergy Immunol. 101:203-208; Rabatic et al., 1993, Exp. Immunol. 94:337-340), isolated from atopic asthmatic individuals. However, until the present discovery, the expression of this receptor on airway smooth muscle cells has not been known.

25 In addition to the discovery of the expression of the FcεRII receptor on airway smooth muscle cells, the present invention includes the discovery that antibodies directed against this receptor block the induction of the asthmatic state. Thus, the invention includes a method of preventing induction of the asthmatic state in

a mammal, which method comprises administering to the mammal a pharmaceutically effective amount of an anti-FcεRII receptor ligand. The ligand binds to the FcεRII receptor protein, thereby inhibiting binding of IgE to the FcεRII receptor protein. Inhibition of binding of IgE to the FcεRII receptor protein serves to prevent induction of the asthmatic state.

The invention also includes a method of treating asthma in a mammal, which method comprises administering to the mammal a pharmaceutically effective amount of an anti-FcεRII receptor ligand. The ligand binds to the FcεRII receptor protein, thereby inhibiting binding of IgE to the FcεRII receptor protein. Inhibition of binding of IgE to the FcεRII receptor protein serves to diminish or ablate the asthmatic state in the mammal thereby treating asthma in the mammal. Preferably, the mammal is a human.

There are two types of FcεRII receptor proteins, FcεRIIa and FcεRIIb, which differ in only six to seven N-terminal amino acids. Both receptor proteins are derived from the same gene through the use of different promoters which control expression of separate first exons of the protein, which exons are spliced to a common mRNA sequence (Delespease et al., 1992, Immunol. Rev. 125:78-97). The invention should be construed to include both receptor proteins.

By the term "asthmatic state" as used herein, is meant the proasthmatic phenotype which is observed in airway smooth muscle cells. This phenotype is characterized by increased contraction and decreased relaxation of the airway tissue when it has been exposed to high IgE-containing atopic asthmatic serum or exogenous IgE, compared with airway tissue which has not been exposed to IgE.

By the term "treating asthma" is meant curing asthma, causing the symptoms of asthma to diminish, ablating or otherwise alleviating the disease.

The invention should be construed to include any ligand that is currently either known or is heretofore unknown, which when bound to an FcεRII receptor protein on an airway smooth muscle cell of a mammal serves to alleviate an asthmatic state in the mammal.

By the term "ligand" as used herein, is meant any natural or synthetic composition or compound which is capable of specifically binding to its cognate receptor protein, and when so bound, prevents binding of IgE to the cognate receptor protein, such that an asthmatic state is prevented or diminished. By way of example, an antibody which specifically binds to an FcεRII receptor protein on an airway smooth muscle cell and inhibits binding of IgE thereto, is termed an "anti-FcεRII receptor protein ligand." In this context, the FcεRII receptor protein is the "cognate receptor protein" for the ligand.

By the term "anti-FcεRII receptor protein ligand" as used herein, is meant any natural or synthetic composition or compound which is capable of binding to an FcεRII receptor protein on an airway smooth muscle cell, which binding prevents binding of IgE to the cognate FcεRII receptor protein.

Preferably, the anti-FcεRII receptor protein ligand is an antibody.

The invention should not be construed to be limited to the specific ligands and respective cognate receptor proteins disclosed in the Examples presented herein. Rather, the invention should be construed to include any presently known or heretofore unknown ligands which have the effect of inhibiting binding of an IgE molecule to an FcεRII receptor protein on an airway smooth muscle cell. It is a simple matter, upon reading the present disclosure, to use the IgE binding inhibition assays described in the Examples to identify additional ligands which bind to an FcεRII receptor protein and inhibit the binding of IgE thereto. Thus, while preferred ligands for use in the methods of the invention are antibodies directed to an FcεRII receptor protein, yet other useful ligands may be identified using the protocols described herein. The most preferred ligand for use in the methods of the invention is an antibody which is an anti-FcεRII receptor protein ligand.

To identify a ligand capable of binding to an FcεRII receptor protein expressed on an airway smooth muscle cell and inhibiting binding of IgE thereto, a mixture comprising a population of cells, for example, airway smooth muscle cells which express an FcεRII receptor protein, and IgE is incubated in the presence or

absence of a test ligand. Binding of IgE to the cells is then assessed. The level of binding of IgE to cells incubated in the presence of the test compound is compared with the level of binding of IgE to cells in the absence of the test compound. A lower level of IgE binding to cells in the presence of the test compound compared with the level of binding of IgE to the cells in the absence of the test compound is an indication that the test compound is an anti-FcεRII receptor protein ligand. Additional assays which definitively establish that the test compound is capable of binding to an FcεRII receptor protein and preventing the binding of IgE thereto, may then be conducted following the protocols provided herein in the Examples section.

The ligand for use in the method of the invention may be any natural or synthetic composition or compound which when bound to its cognate receptor protein, effects the inhibition of binding of IgE to the cognate receptor protein. Thus, the ligand may be a protein, a peptide or a small molecule. The ligand may be administered to a cell as is, that is, as an isolated protein, an isolated peptide, a small molecule, or it may be administered to the cell in the form of an isolated nucleic acid sequence encoding the ligand.

By the term "isolated nucleic acid", as used herein, refers to a nucleic acid sequence, a DNA or an RNA or fragment thereof which has been separated from the sequences which flank it in a naturally occurring state, *e.g.*, a DNA fragment which has been removed from the sequences which are normally adjacent to the fragment, *e.g.*, the sequences adjacent to the fragment in a genome in which it naturally occurs. The term also applies to nucleic acids which have been substantially purified from other components which naturally accompany the nucleic acid, *e.g.*, RNA or DNA or proteins, which naturally accompany it in the cell. The term therefore includes, for example, a recombinant DNA which is incorporated into a vector; into an autonomously replicating plasmid or virus; or into the genomic DNA of a prokaryote or eukaryote; or which exists as a separate molecule (*e.g.*, as a cDNA or a genomic or cDNA fragment produced by PCR or restriction enzyme digestion) independent of

other sequences. It also includes a recombinant DNA which is part of a hybrid gene encoding additional polypeptide sequences.

5 In other related aspects, the invention includes vectors which contain such isolated nucleic acid and which are preferably capable of directing expression of the protein encoded by the nucleic acid in a vector-containing cell; and cells containing such vectors, either eukaryotic cells or prokaryotic cells, preferably eukaryotic cells.

10 The isolated nucleic acid of the invention should be construed to include an RNA or a DNA sequence encoding a ligand of the invention, and any modified forms thereof, including chemical modifications of the DNA or RNA which render the nucleotide sequence more stable when it is cell free or when it is associated with a cell. Chemical modifications of nucleotides may also be used to enhance the efficiency with which a nucleotide sequence is taken up by a cell or the efficiency with which it is expressed in a cell. Any and all combinations of modifications of the nucleotide sequences are contemplated in the present invention.

15 By the terms "isolated peptide" or "isolated protein," as used herein, is meant a peptide or protein which has been substantially separated from the components, e.g., DNA, RNA, other proteins and peptides, carbohydrates and lipids, which naturally accompany the protein or peptide in the cell. The terms isolated peptide and protein may be construed to include a peptide or protein which is expressed and/or secreted from a cell comprising an isolated nucleic acid.

20 The present invention also provides for analogs of proteins or peptides which comprise a ligand as defined herein. Analogs may differ from naturally occurring proteins or peptides by conservative amino acid sequence differences or by modifications which do not affect sequence, or by both. For example, conservative amino acid changes may be made, which although they alter the primary sequence of the protein or peptide, do not normally alter its function. Conservative amino acid substitutions typically include substitutions within the following groups:

glycine, alanine;
valine, isoleucine, leucine;

aspartic acid, glutamic acid;
asparagine, glutamine;
serine, threonine;
lysine, arginine;
5 phenylalanine, tyrosine.

Modifications (which do not normally alter primary sequence) include *in vivo*, or *in vitro* chemical derivatization of polypeptides, *e.g.*, acetylation, or carboxylation. Also included are modifications of glycosylation, *e.g.*, those made by modifying the glycosylation patterns of a polypeptide during its synthesis and processing or in further
10 processing steps; *e.g.*, by exposing the polypeptide to enzymes which affect glycosylation, *e.g.*, mammalian glycosylating or deglycosylating enzymes. Also embraced are sequences which have phosphorylated amino acid residues, *e.g.*, phosphotyrosine, phosphoserine, or phosphothreonine.

Also included are polypeptides which have been modified using
15 ordinary molecular biological techniques so as to improve their resistance to proteolytic degradation or to optimize solubility properties or to render them more suitable as a therapeutic agent. Analogs of such polypeptides include those containing residues other than naturally occurring L-amino acids, *e.g.*, D-amino acids or non-naturally occurring synthetic amino acids. The peptides of the invention are not limited to
20 products of any of the specific exemplary processes listed herein.

Desirable isolated protein or isolated peptide ligands include antibodies which bind to the desired cognate receptor protein. The antibody may be any type of antibody including, but not limited to, a polyclonal antibody, a monoclonal antibody, a synthetic antibody, a chimeric antibody, a humanized antibody, and the like. Other
25 anti-FcεRII receptor protein ligands include proteins which are not antibodies, such as

Antibody technology is described in Harlow et al. (1988, In: Antibodies, A Laboratory Manual, Cold Spring Harbor, NY). Polyclonal antibodies directed against an FcεRII receptor protein may be made by immunizing any suitable animal

and obtaining immune serum from the animal at selected intervals following immunization.

Monoclonal antibodies directed against full length or peptide fragments of an FcεRII receptor protein may be prepared using any well known monoclonal antibody preparation procedures, such as those described, for example, in Harlow et al. (supra). Quantities of the desired peptide may also be synthesized using chemical synthesis technology. Alternatively, DNA encoding the desired peptide may be cloned and expressed from an appropriate promoter/regulatory sequence in cells which are suitable for the generation of large quantities of peptide. Monoclonal antibodies directed against the peptide are generated from mice immunized with the peptide using standard procedures as referenced herein.

Nucleic acid encoding the monoclonal antibody obtained using the procedures described herein may be cloned and sequenced using technology which is available in the art, and is described, for example, in Wright et al. (1992, Critical Rev. in Immunol. 12(3,4):125-168) and the references cited therein. Further, the antibody may be "humanized" using the technology described in Wright et al., (supra) and in the references cited therein.

To generate a phage antibody library, a cDNA library is first obtained from mRNA which is isolated from cells, e.g., the hybridoma, which express the desired protein to be expressed on the phage surface, e.g., the desired antibody. cDNA copies of the mRNA are produced using reverse transcriptase. cDNA which specifies immunoglobulin fragments are obtained by PCR and the resulting DNA is cloned into a suitable bacteriophage vector to generate a bacteriophage DNA library comprising DNA specifying immunoglobulin genes. The procedures for making a bacteriophage library comprising heterologous DNA are well known in the art and are described, for example, in Sambrook et al. (1989, Molecular Cloning. A Laboratory Manual, Cold Spring Harbor, NY) and in Ausubel et al. (Ausubel et al., 1993, Current Protocols in Molecular Biology, Green & Wiley, New York).

Bacteriophage which encode the desired antibody, may be engineered such that the protein is displayed on the surface thereof in such a manner that it is available for binding to its corresponding binding protein, *e.g.*, the antigen against which the antibody is directed. Thus, when bacteriophage which express a specific
5 antibody are incubated in the presence of a cell which expresses the corresponding antigen, the bacteriophage will bind to the cell. Bacteriophage which do not express the antibody will not bind to the cell. Such panning techniques are well known in the art and are described for example, in Wright et al., (*supra*).

Processes such as those described above, have been developed for the
10 production of human antibodies using M13 bacteriophage display (Burton et al., 1994, *Adv. Immunol.* 57:191-280). Essentially, a cDNA library is generated from mRNA obtained from a population of antibody-producing cells. The mRNA encodes rearranged immunoglobulin genes and thus, the cDNA encodes the same. Amplified cDNA is cloned into M13 expression vectors creating a library of phage which express
15 human Fab fragments on their surface. Phage which display the antibody of interest are selected by antigen binding and are propagated in bacteria to produce soluble human Fab immunoglobulin. Thus, in contrast to conventional monoclonal antibody synthesis, this procedure immortalizes DNA encoding human immunoglobulin rather than cells which express human immunoglobulin.

20 The procedures just presented describe the generation of phage which encode the Fab portion of an antibody molecule. However, the invention should not be construed to be limited solely to the generation of phage encoding Fab antibodies. Rather, phage which encode single chain antibodies (scFv/phage antibody libraries) are also included in the invention. Fab molecules comprise the entire Ig light chain, that is,
25 they comprise both the variable and constant region of the light chain, but include only the variable region and first constant region domain (CH1) of the heavy chain. Single chain antibody molecules comprise a single chain of protein comprising the Ig Fv fragment. An Ig Fv fragment includes only the variable regions of the heavy and light chains of the antibody, having no constant region contained therein. Phage libraries

comprising scFv DNA may be generated following the procedures described in Marks et al. (1991, J. Mol. Biol. 222:581-597). Panning of phage so generated for the isolation of a desired antibody is conducted in a manner similar to that described for phage libraries comprising Fab DNA.

5 The invention should also be construed to include synthetic phage display libraries in which the heavy and light chain variable regions may be synthesized such that they include nearly all possible specificities (Barbas, 1995, Nature Medicine 1:837-839; de Kruif et al. 1995, J. Mol. Biol. 248:97-105).

10 By the term "synthetic antibody" as used herein, is meant an antibody which is generated using recombinant DNA technology, such as, for example, an antibody expressed by a bacteriophage as described herein. The term should also be construed to mean an antibody which has been generated by the synthesis of a DNA molecule encoding the antibody and which DNA molecule expresses an antibody protein, or an amino acid sequence specifying the antibody, wherein the DNA or amino
15 acid sequence has been obtained using synthetic DNA or amino acid sequence technology which is available and well known in the art.

 The invention thus includes an isolated DNA encoding an anti-FcεRII receptor protein ligand or DNA encoding a portion of the ligand, which when the ligand is, for example, an antibody, the antibody is itself specific for its cognate
20 receptor protein, or for fragments thereof.

 To isolate DNA encoding an antibody, for example, DNA is extracted from antibody expressing phage obtained as described herein. Such extraction techniques are well known in the art and are described, for example, in Sambrook et al. (supra) and in Ausubel et al. (supra).

25 By the term "scFv/phage" are used herein, is meant a phage particle which expresses the Fv portion of an antibody as a single chain.

 The invention should be construed to include other anti-FcεRII receptor protein-binding ligands which are either known or are heretofore unknown, which are,

or will be designed to bind to an FcεRII receptor protein and which are useful for treating or preventing the asthmatic state in an individual.

Another form of ligand includes a nucleic acid sequence which encodes the anti-FcεRII receptor protein ligand and which is associated with
5 promoter/regulatory sequences which can direct expression of the anti-FcεRII receptor protein ligand *in vivo*. For a discussion of this technology, see, for example, Cohen (1993, Science 259:1691-1692), Fynan et al. (1993, Proc. Natl. Acad. Sci. 90:11478-11482) and Wolff et al. (1991, Biotechniques 11:474-485) which describe similar the use of naked DNA as a therapeutic agent. For example, a plasmid containing suitable
10 promoter/regulatory sequences operably linked to a DNA sequence encoding an anti-FcεRII receptor protein ligand, may be directly administered to a patient using the technology described in the aforementioned references.

As used herein, the term "promoter/regulatory sequence" means a DNA sequence which is required for tissue-specific, organ specific, or other specific (such as
15 inducible, etc) expression of a gene operably linked to the promoter/regulator sequence. In some instances, this sequence may be the core promoter sequence and in other instances, this sequence may also include an enhancer sequence and other regulatory elements which are required for expression of the gene in the desired specific manner.

By describing two nucleic acid sequences as "operably linked" as used
20 herein, is meant that a single-stranded or double-stranded nucleic acid moiety comprises each of the two nucleic acid sequences and that the two sequences are arranged within the nucleic acid moiety in such a manner that at least one of the two nucleic acid sequences is able to exert a physiological effect by which it is characterized upon the other.

25 Alternatively, the promoter/enhancer sequence operably linked to DNA encoding the anti-FcεRII receptor protein ligand may be contained within a vector, which vector is administered to the patient. The vector may be a viral vector which is suitable as a delivery vehicle for delivery of the anti-FcεRII receptor protein ligand DNA to the patient, or the vector may be a non-viral vector which is suitable for the

same purpose. Examples of viral and non-viral vectors for delivery of DNA to cells and tissues are well known in the art and are described, for example, in (Ma et al., 1997, Proc. Natl. Acad. Sci. USA 94:12744-12746). Examples of viral vectors include, but are not limited to, a recombinant vaccinia virus, a recombinant adenovirus, a recombinant retrovirus, a recombinant adeno-associated virus, a recombinant avian pox virus, and the like (Cranage et al., 1986, EMBO J. 5:3057-3063; International Patent Application No. WO94/17810, published August 18, 1994; International Patent Application No. WO94/23744, published October 27, 1994). Examples of non-viral vectors include, but are not limited to, liposomes, polyamine derivatives of DNA, and the like.

The identity, selection and means for obtaining a desired anti-FcεRII receptor protein ligand useful for treatment or prevention of asthma may be performed by the skilled artisan using conventional technology when in possession of the present invention. For example, as described in the Examples presented herein, there are a variety of anti-FcεRII monoclonal antibodies which are available that are likely anti-FcεRII receptor protein ligands suitable for use in the methods of the invention.

Other anti-FcεRII receptor protein ligands may include, but are not limited to, isolated proteins and isolated polypeptides and isolated nucleic acid sequences encoding the same. Isolated proteins and peptides having anti-FcεRII receptor protein ligand activity and isolated nucleic acids encoding the same, may be chemically synthesized by conventional methods known in the art, or they may be purchased for a commercial source if available. In one embodiment of the invention, the anti-FcεRII receptor protein ligand, being a protein, a peptide or a nucleic acid, may be produced using recombinant techniques *in vitro* in sufficiently large quantities for use in a therapeutic composition for use in treating or preventing asthma. In addition, a recombinant virus vector comprising DNA encoding the desired anti-FcεRII receptor protein ligand may be prepared using conventional recombinant DNA technology procedures.

The ligand useful in the methods of the invention may be a small molecule, a non-peptide, a peptidomimetic, and the like, which ligand binds to an FcεRII receptor protein thereby inhibiting binding of IgE to the receptor protein. Once in possession of the present invention, it is within the skill of the ordinary artisan to identify the contact points between the ligand and the cognate receptor protein, which contact points are essential for binding of these molecules together to inhibit binding of IgE to the receptor protein. Thus, it is also within the skill of the artisan to design specific peptidomimetics which bind to FcεRII receptor protein. The invention should be construed to include such peptidomimetics. The technology of the development of peptidomimetics is described, for example, in PCT/US93/01201 and U.S. Patent No. 5,334,702.

The anti-FcεRII receptor protein ligand of the invention may be formulated in a pharmaceutical composition which is suitable for administration of the ligand to a human patient. It will be appreciated that the precise formulation and dosage amounts will vary depending upon any number of factors, including, but not limited to, the type and severity of the disease to be treated, the route of administration, the age and overall health of the individual, the nature of the ligand, etc. However, the preparation of a pharmaceutically acceptable composition having an appropriate pH, isotonicity, stability and other characteristics is within the skill of the art. Pharmaceutical compositions are described in the art, for example, in Remington's Pharmaceutical Sciences (Genaro ed., 1985, Mack Publishing Co., Easton, PA).

As used herein, the term "pharmaceutically-acceptable carrier" means a chemical composition with which an appropriate anti-FcεRII receptor protein ligand, may be combined and which, following the combination, can be used to administer the ligand to a patient.

The amount of the anti-FcεRII receptor protein ligand composition administered, whether it is administered as protein or as nucleic acid, is sufficient to prevent, diminish or alleviate the asthmatic state. The pharmaceutical compositions useful for practicing the invention may be administered to deliver a dose of between

about 1 ng/kg and about 100 mg/kg of patient body weight. Suitable amounts of the anti-FcεRII receptor protein ligand for administration include doses which are high enough to have the desired effect without concomitant adverse effects. When the anti-FcεRII receptor protein ligand is a protein or peptide, a preferred dosage range is from about 10 to about 1000 μg of protein or peptide per kg of patient body weight. When the anti-FcεRII receptor protein ligand is administered in the form of DNA encoding the same contained within a recombinant virus vector, a dosage of between about 10² and about 10¹¹ plaque forming units of virus per kg of patient body weight may be used. When naked DNA encoding the anti-FcεRII receptor protein ligand is to be administered as the pharmaceutical composition, a dosage of between about 10 μg about several mg of DNA per kg of patient body weight may be used.

In the practice of the methods of the invention, a composition containing an anti-FcεRII receptor protein ligand is administered to a patient in a sufficient amount to prevent, diminish or alleviate an asthmatic state in the individual. Patients to be treated include children and adults who have atopic (allergic) asthma. This constitutes the vast majority of asthmatic individuals.

The frequency of administration of an anti-FcεRII receptor protein ligand to a patient will also vary depending on several factors including, but not limited to, the type and severity of the asthma to be treated, the route of administration, the age and overall health of the individual, the nature of the anti-FcεRII receptor protein ligand, etc. It is contemplated that the frequency of administration of the anti-FcεRII receptor protein ligand to the patient may vary from about once every few months to about once a month, to about once a week, to about once per day, to about several times daily.

Pharmaceutical compositions that are useful in the methods of the invention may be administered systemically in parenteral, oral solid and liquid formulations, ophthalmic, suppository, aerosol, topical or other similar formulations. In addition to the appropriate anti-FcεRII receptor protein ligand, these pharmaceutical compositions may contain pharmaceutically-acceptable carriers and other ingredients

known to enhance and facilitate drug administration. Thus such compositions may optionally contain other components, such as adjuvants, e.g., aqueous suspensions of aluminum and magnesium hydroxides, and/or other pharmaceutically acceptable carriers, such as saline. Other possible formulations, such as nanoparticles, liposomes, resealed erythrocytes, and immunologically based systems may also be used to administer the appropriate anti-FcεRII receptor protein ligand to a patient according to the methods of the invention.

Preferably, the composition of the invention is administered to the human by a lung inhalation route, i.e., via a nebulizer or other lung inhalation device.

An anti-FcεRII receptor protein ligand may be administered in conjunction with other compounds which are used to treat asthma. Such compounds include, but are not limited to, corticosteroids, sodium cromolyn, methylxanthines, leukotriene modifiers), anti-cholinergic agents, and rapid relief medications that counteract bronchospasm, e.g., primarily beta-adrenergic agents. The choice of which additional compound to administer will vary depending upon any number of the same types of factors that govern the selection of dosage and administration frequency of the anti-FcεRII receptor protein ligand. Selection of these types of compounds for use in conjunction with an anti-FcεRII receptor protein ligand for practice of the method of the invention is well within the skill of those in the art.

The invention will be further described by reference to the following experimental examples. These examples are provided for purposes of illustration only, and are not intended to be limiting unless otherwise specified. Thus, the invention should in no way be construed as being limited to the following examples, but rather, should be construed to encompass any and all variations which become evident as a result of the teaching provided herein.

Examples

The experiments presented were designed to examine whether ASM cells have the capacity to intrinsically express Fc receptors. The experiments were also designed to examine whether the expression and activation of these Fc receptors

is altered in the atopic asthmatic sensitized state, which alteration may contribute to changes in agonist responsiveness of the ASM tissue. The results demonstrate that a) enhanced constrictor and attenuated relaxation responsiveness induced in ASM passively sensitized with human atopic asthmatic serum are prevented by depleting the sensitizing serum of its immune complexes or by blockade of FcεRII activation with an anti-CD23 blocking antibody; b) exposure of ASM cells and tissue to atopic asthmatic serum induces autologously upregulated mRNA and cell surface expression of FcεRII receptors by the ASM and; c) the latter induced upregulated expression of the FcεRII receptor in the atopic sensitized state is ablated by an anti-CD23 blocking antibody. Taken together, these observations provide new evidence that airway smooth muscle cells intrinsically express Fc receptors and that the induced altered responsiveness of atopic asthmatic sensitized airway smooth muscle is largely attributed to its autologously upregulated expression and activation of the FcεRII receptor subtype. Notwithstanding the well-established contribution of airway infiltrating inflammatory cells in the pathogenesis of atopic asthma (Beasley et al., 1989, *Am. Rev. Respir. Dis.* 139:806-817; Litchfield et al., 1992, *J. Asthma* 29:181-191; Barnes et al., 1988, *Pharmacol. Rev.* 40:49-84), the present novel findings identify a critical role for the airway smooth muscle itself in autologously regulating IgE/CD23-coupled changes in airway reactivity which characterize the asthmatic state.

The data may be summarized as follows. To elucidate the role of immunoglobulin (Ig) E-dependent mechanisms in inducing altered airway responsiveness in the atopic asthmatic state, the expression and actions of Fc receptor activation were examined in isolated rabbit tracheal smooth muscle (TSM) tissue and cultured cells passively sensitized with sera from atopic asthmatic patients or nonatopic/nonasthmatic (control) subjects. Relative to control tissues, the atopic asthmatic-sensitized TSM exhibited significantly increased maximal isometric contractility to acetylcholine ($p < 0.01$) and attenuated maximal relaxation responses and sensitivity (i.e., $-\log ED_{50}$) to isoproterenol ($p < 0.005$). These changes in agonist

responsiveness in atopic sensitized TSM were ablated by pretreating the tissues with a monoclonal blocking antibody (MAb) to the low affinity receptor for IgE, FcεRII (i.e., CD23) or by depleting the sensitizing serum of its immune complexes. Moreover, in complimentary experiments, exogenous administration of IgE immune complexes to
5 naive TSM produced changes in agonist responsiveness which were qualitatively similar to those obtained in the atopic asthmatic sensitized state. Additional experiments established that, in contrast to their respective controls, atopic asthmatic serum-sensitized human and rabbit TSM tissue and cultured cells exhibited markedly induced mRNA and cell surface expression of FcεRII, whereas constitutive expression
10 of the IgG receptor subtype, FcγRIII, was unaltered. Moreover, the upregulated mRNA expression of FcεRII observed following exposure of TSM to atopic asthmatic serum or to exogenously administered IgE immune complexes was significantly inhibited by pretreating the tissues or cells with anti-CD23 MAb. Collectively, these data provide new evidence demonstrating that the altered agonist responsiveness in
15 atopic asthmatic sensitized airway smooth muscle is largely attributed to IgE-mediated induction of the autologous expression and activation of FcεRII receptors in the airway smooth muscle itself.

The Materials and Methods used in the experiments presented herein are now described.

20 Animals

Thirty four adult New Zealand White rabbits were used in the present study which was approved by the Biosafety and Animal Research Committee of the Joseph Stokes Research Institute at The Children's Hospital of Philadelphia. The animals exhibited no signs of respiratory disease for several weeks before the study.

25 Preparation and sensitization of airway smooth muscle tissue

Rabbits were anesthetized with xylazine (10 mg/kg) and ketamine (50 mg/kg) and were subsequently sacrificed using an overdose of pentobarbital (130 mg/kg). The tracheae of the rabbits were removed via open thoracotomy, the tracheae were cleared of loose connective tissue and were divided into eight ring segments of 6-

8 mm length. Each segment referred to herein as a tracheal smooth muscle (TSM) segment was incubated for 24 hours at room temperature in either of the following: 1) human serum containing immunoglobulin (Ig) E at greater than 1000 IU/ml which was obtained from allergic patients having moderate to severe asthma and a 4-5/6+ radioallergosorbent test (RAST) positive (having a specific IgE concentration of more than 17.5 Phadebas RAST units (PRU)/ml) to *Dermatophagoides pteronyssimus*, *Dermatophagoides farinae* and ragweed, and a positive skin test to these antigens; or 2) human serum obtained from non-atopic, non-asthmatic individuals having normal serum IgE levels (i.e., < 70 IU/ml) and negative skin test reactivity to *Dermatophagoides pteronyssimus*, *Dermatophagoides farinae* and ragweed (Hakonarson et al., 1995, Am. J. Physiol. (Lung Cell Mol. Physiol.) 269:L645-L652). In parallel experiments, TSM segments were incubated in either control serum or in atopic asthmatic serum that was: a) depleted of its immunoglobulin complexes by pretreatment with sepharose Staphylococcus protein A as described (Kessler, 1975, J. Immunol. 115:1617-1624); b) co-incubated with an anti-CD23 (40 µg/ml) monoclonal blocking antibody; or c) with an anti-CD16 (2 µg/ml) monoclonal blocking antibody. The TSM segments were treated with their respective anti-CD23 or anti-CD16 antibodies in Dulbecco's modified Eagle's medium for 1 hour prior to exposure to the atopic asthmatic serum. In extended experiments, paired TSM segments were incubated for 24 hours in Dulbecco's modified Eagle's medium, as previously described (Hakonarson et al., 1996, J. Clin. Invest. 97:2593-2600), containing either: a) human IgE (final bath concentration (FBC): 15 µg/ml); or b) human IgE-goat-anti-human IgE immune complexes (FBC: 15:5 µg/ml of IgE/anti-IgE in the final organ bath mixture). Tissues incubated in medium alone served as controls. The concentrations of immune complexes used for the TSM incubations were based on the results obtained in pilot studies which were designed to determine the concentration and ratio of immune complexes that induced the greatest acute contractile effect in isolated sensitized TSM segments. The serum was aerated with a continuous supplemental O₂ mixture (95% O₂/5% CO₂) during the incubation phase.

In comparable experiments, the passive sensitization protocol described above was also conducted on human tracheal smooth muscle which was isolated 1 hour post-mortem from a 53 year-old male who died from a central nervous system complication of immune thrombocytopenia and had no evidence of lung disease. The passive sensitization protocol was also conducted on a surgically resected human bronchial smooth muscle segment obtained from a 72 year-old female with peripheral lung carcinoma. Microscopically normal airway smooth muscle was carefully cleaned of loose connective tissue and epithelium and divided into 4-5 mm long strips. As above, each alternate adjacent strip was incubated in either control or atopic asthmatic serum in the absence or presence of an anti-CD23 antibody and was subsequently examined for Fc receptor expression (see below).

Preparation and sensitization of airway smooth muscle cells

Airway smooth muscle (ASM) cells were cultured according to described protocols. These cells have been characterized in detail with respect to their distinguishing morphological, histological, and immunological features. The cell isolation and subcultivation procedures, and the characterization of the cells is described in Noveral et al. (1992, Am. J. Physiol. (Lung Cell. Mol. Physiol.) 263:L555-L561). Briefly, ASM cells were isolated from epithelium-denuded trachealis muscle obtained from adult New Zealand White rabbits. Following digestion in F-12 culture medium containing 30 mg/ml protease, 55 mg/ml type IV collagenase, and 100 mg/ml trypsin inhibitor, the dissociated cells were centrifuged and resuspended in F-12 containing 10% fetal bovine serum (FBS) and 100 µg/ml of gentamicin sulfate. The cells were then inoculated in 100 mm tissue culture dishes and, after 4 weeks, the cells had sufficiently proliferated to permit routine subcultivations. At weekly intervals, the subcultivated cells were suspended and then inoculated at a density of 1×10^4 cells/cm² in 75 cm² tissue culture flasks containing F-12 supplemented with 20% FBS and were then incubated at 37°C in a humidified atmosphere of 5% CO₂/95% air. Routine tests for mycoplasma contamination were negative. For the sensitization protocol, cells initially seeded at 1×10^4 cells/cm² in 75-

cm² tissue culture flasks were grown to confluence in Ham's F-12 medium containing 20% FBS. The original culture medium was then replaced with Ham's F-12 for 24 hours and was subsequently changed to F-12 containing either of the following: 1) 10% human serum containing immunoglobulin (IgE) E at greater than 1000 IU/ml obtained from allergic patients with moderate to severe asthma (as above); 2) 10% human serum obtained from non-atopic non-asthmatic (control) individuals (Hakonarson et al., 1996, J. Clin. Invest. 97:2593-2600); 3) IgE immune complexes (15:5 µg/ml), or; 4) F-12 alone, for the various time points.

Pharmacodynamic studies

Following incubation of the tissue preparations obtained as described herein, each rabbit airway segment was suspended longitudinally between stainless steel triangular supports in siliconized Harvard 20 ml organ baths. The lower support was secured to the base of the organ bath, and the upper support was attached via a gold chain to a Grass FT.03C force transducer from which isometric tension was continuously displayed on a multichannel recorder. Care was taken to place the membranous portion of the trachea between the supports in order to maximize the recorded tension generated by the contracting trachealis muscle. The tissues were bathed in modified Krebs-Ringer solution containing 125 mM NaCl, 14 mM NaHCO₃, 4 mM KCl, 2.25 mM CaCl₂·H₂O, 1.46 MgSO₄·H₂O, 1.2 mM NaH₂PO₄·H₂O and 11 mM glucose. The baths were aerated with 5% CO₂ in oxygen; a pH of 7.35-7.40 was maintained, and the organ bath temperature was held at 37°C. Passive resting tension of each TSM segment was set at 2.0 g after each tissue had been passively stretched to a tension of 8 g in order to optimize the resting length of each segment as described (Tanaka et al., 1990, J. Clin. Invest. 85:345-350). The tissues were allowed to equilibrate in the organ baths for 45 minutes, at which time each tissue was primed with a 1 minute exposure to 10⁻⁴ M acetylcholine (ACh). Cholinergic contractility was subsequently assessed in the TSM segments by cumulative administration of ACh in final bath concentrations ranging from 10⁻¹⁰-10⁻³ M. Thereafter, in separate studies, relaxation dose-response curves to isoproterenol (10⁻¹⁰-10⁻⁴ M) were conducted in

tissues half-maximally contracted with ACh. The relaxant responses to isoproterenol were analyzed in terms of % maximal relaxation (Rmax) from the active cholinergic contraction, and sensitivity to the relaxing agent was determined as the negative logarithm of the dose of the relaxing agent producing 50% of Rmax (pD₅₀) (i.e., geometric mean ED₅₀ value).

Characterization of Fc receptor mRNA by Reverse Transcriptase Polymerase Chain Reaction (RT-PCR)

Total RNA was isolated from sensitized and control ASM cells and tissue using the modified acid guanidinium thiocyanate phenol-chloroform extraction method (Chomczynski et al., 1987, Anal. Biochem. 162:156-159) which includes proteinase K (in 0.5% SDS) digestion of protein in the initial RNA pellet. The concentration of each RNA sample was then determined spectrophotometrically. This procedure consistently yielded 20-25 µg of intact RNA per each T-75 flask of ASM cells.

To analyze mRNA expression specific for the FcγRI, R-IIA, -IIC, -IIB, -RIII, and FcεRII receptor subtypes, an RT-PCR protocol and human-specific primers for these Fc receptor subtypes was used. cDNA was synthesized using 2.5 µg of total RNA isolated from the cell and tissue samples following 0, 6 and 24 hours of treatment with either 10% control or 10% atopic asthmatic human serum in the presence or absence of an anti-CD23 blocking antibody, and using RNA from cells treated with IgE immune complexes or F-12 serum-free medium alone. cDNA generated using RNA obtained from immortalized U937 cells and the B cell line 8.1.6, were used as positive controls for FcγRIII and FcεRII mRNA expression, respectively. The cDNA was primed with oligo (dT)₁₂₋₁₈ and with random hexamer nucleotides (N6) in the absence of signal using the former method. Two µl of cDNA was used in each PCR reaction. The Fc receptor primers used in the PCR assays were based on the published sequences of the human Fcγ -RI, -RII, -RIII and FcεRII genes (Capel et al., 1994, Immunometh. 4:25-34; Kikutani et al., 1986, Cell 47:657-665) and included the following primer sets:

Fcγ RI: 5' Primer: 5'-ATGTGGTTCTTGACAACTCTGCTC 3'
 (SEQ ID NO:1)
 3' Primer: 5'-ATGTCTGTCTTCTTGAAGGCTGGA-3'
 (SEQ ID NO:2) (product is 1,038 bp)

5 Fcγ RIIA,C: 5' Primer: 5'-GACTCCATTCAAGTGGTTCCA-3'
 (SEQ ID NO:3)
 3' Primer: 5'-GTCAGCTGTTTCATAGTCATTG-3'
 (SEQ ID NO:4) (product is 644 bp)

10 Fcγ RIIB: 5' Primer: 5'-GACTCCATTCAAGTGGTTCCA-3'
 (SEQ ID NO:5)
 3' Primer: 5'-CCCAACTTTGTCAGCCTCATC- 3'
 (SEQ ID NO:6) (product is 618 bp)

Fcγ RIII: 5' Primer: 5'-AAGATCTCCCAAAGGCTGTG-3'
 (SEQ ID NO:7)
 15 3' Primer: 5'-ATGGACTTCTAGCTGCACCG-3'
 (SEQ ID NO:8) (product is 254 bp)

FcεRII: 5' Primer: 5'-CGTCTCTCAAGTTTCCAAG-3'
 (SEQ ID NO:9)
 3' Primer: 5'-GCACTTCCGTTGGGAATTTG-3'
 20 (SEQ ID NO:10) (product is 333 bp)

Rabbit specific α-actin primers, 5'-CGACATCAAGGAGAAGCTG-3'
 (SEQ ID NO:11) and 5'-CTAGAAGCATTTGCGGTGC-3' (SEQ ID NO:12) (19
 mers), and human specific ribosomal protein L7 (RPL7) primers 5'-
 AAGAGGCTCTCATTTTCCTGGCTG-3' (SEQ ID NO:13) (24 mer) and 5'-
 25 TCCGTTCTCCTCCCAATAATGTTCC-3' (SEQ ID NO:14) (23 mer), based on the
 published sequence of the rabbit α-actin (Putney et al., 1983, Nature 302:718-721)
 and human RPL7 genes (Seshadri et al., 1993, J. Biol. Chem. 268:18474-18480),
 respectively, were used to assess general transcription levels in each sample. The
 cycling profile used was as follows: Denaturation: 95° C for 1 minute; annealing: 54°

C for 1 minute; and extension: 72° C for 1-2 minutes using 40 cycles for the Fcγ RI, RII, RIII and Fcε RII genes; and using 22 cycles for the α-actin and RPL7 genes, respectively. The number of cycles was determined to be in the linear range of production of the PCR product. The PCR reactions using the human Fcγ, Fcε, RPL7, and rabbit α-actin primers were performed using equivalent amounts of cDNA prepared from 2.5 μg of total RNA. Equal aliquots of each PCR reaction were then run on a 1.2% agarose gel and were subsequently transferred to a Zeta-probe membrane overnight in 0.4 N NaOH. Following capillary transfer, the DNA was immobilized by UV-crosslinking using a Stratilinker UV Crosslinker 2400 at 120,000 microjoules/cm² (Stratagene).

Prehybridization in a Techne hybridization oven was conducted for 2-3 hr at 42° C in 50% formaldehyde, 7% (w/v) SDS, 0.25 M NaCl, 0.12 M Na₂HPO₄ (pH 7.2), and 1 mM EDTA. Hybridization was for 20 hr at 42°C in the same solution. The Fcγ RI, RII, RIII; Fcε RII; RPL7 and α-actin DNA levels were assayed by Southern blot analysis using ³²P-labeled probes. The FcγRIII, α-actin and RPL7 probes were prepared by pooling several RT-PCR reactions which used the FcγRIII, α-actin and RPL7 PCR fragments and purifying them from a 1.2% agarose gel using Qiaex II agarose gel extraction kit. The FcγRIII, α-actin and RPL7 cDNA fragments were subsequently sequenced for product confirmation. The other individual human Fcγ and Fcε probes were obtained from the cloned cDNA sequences of these genes. In addition, the 333 bp rabbit ASM CD23 RT-PCR products were also sequenced for product confirmation, and exhibited ~90% homology with the human CD23 B-lymphocyte receptor gene. Washes were as follows: 1 x 15 minutes in 2 X SSC, 0.1% SDS; 1 x 15 minutes in 0.1 X SSC, 0.1% SDS both at room temperature, and 2 x 15 minutes at 50° C in 0.1 X SSC, 0.1% SDS. Southern blots were quantitated by direct measurements of radioactivity in each band using a Phosphorimager (Molecular Dynamics).

Determination of Fc receptor expression in ASM cells and tissue

Fc receptor cell surface expression was examined in cultured rabbit ASM cells using a Coulter EPICS Elite flow cytometer (Coulter EPICS Division, Hialeah, FL) equipped with a 5 watt argon laser operated at 488 nM and 300 mwatt output. Fluorescence signals were accumulated as two parameter fluorescence histograms wherein both % positive cells and mean channel fluorescence were recorded. Cells which were treated for 24 hours with either 10% atopic asthmatic or 10% control human serum were resuspended in buffer, dispersed by passage through a 23 g needle, and then stained with the individual antibodies. Based on the results of the Fc receptor mRNA expression studies, the targeted monoclonal antibodies used included the 3G8-FITC anti-FcγRIII (Medarex, Inc., Annandale, NJ) and the FITC mouse monoclonal antibody to human CD23 (Calteg, San Francisco, CA). The immortalized B-cell line 8.1.6, was used as the positive control for the CD23 receptor expression assay. The cells were also stained with FITC- and PE- conjugated mouse antibodies having the identical isotypes as the Fc receptor monoclonals to measure background fluorescence (i.e., IgG3-FITC as control for anti-CD23; and IgG₂a-PE as control for FcγRIII, respectively). The antibody- stained cells were then evaluated by flow cytometry and were analyzed using the Elite Immuno 4 statistical software (Coulter EPICS Division, Hialeah, FL). Fluorescence intensities were expressed as % positive cells, including mean channel fluorescence.

An immunofluorescence detection assay was also used to examine for FcεRII surface receptor expression in rabbit ASM cells and tissue following 24 hours of treatment with human control vs. atopic asthmatic serum, as described above. The ASM cells were fixed in acetone alone, whereas the tissues were embedded in OCT compound (Miles Laboratories) and frozen in acetone/dry ice. Serial 3-5 μm TSM sections were prepared and mounted on poly-L-lysine coated slides. The specimens were incubated with PBS buffer containing 10% rabbit serum to suppress non-specific staining and were subsequently labeled overnight at 4° C with primary mouse anti-human CD23 (FcεRII) antibody at a 1:250-500 dilution. In control sections, the primary antibody was replaced by immunoglobulins of the same isotype following the

manufacture's protocol (mouse IgG1 negative control). Parallel cell and tissue slides were also stained with an α -actin antibody. After subsequent repeat washing, FITC-labeled F(ab')₂ goat anti-mouse IgE (Fce) fragments was added as the secondary antibody. The mixtures were incubated for 1 hour in 1:500-1000 dilutions in PBS containing 0.5% BSA. After serial washing, the slides were examined using a fluorescent microscope, and quantitative analysis of the protein localization in the cell and tissue sections under the above experimental conditions was performed using the Metamorph Imaging System interfaced with a Nikon Diaphot 300 Image Analyzer that utilizes a Hamamatsu CCD camera.

Statistical analysis

Unless otherwise indicated, results are expressed as mean \pm SE.

Statistical analysis was performed by means of two-tailed paired Student's t-test. P-values < 0.05 were considered significant.

Reagents

The Fc γ -RI, -RII, -RIII, Fc ϵ RII, RPL7 and rabbit α -actin primers were obtained from Integrated DNA Technologies Inc., Coralville, IA. ACh and isoproterenol hydrochloride were obtained from Sigma Chemical, St. Louis, MO. The human IgG, goat-antihuman IgG, human myeloma IgE, and the goat-antihuman IgE antibodies were purchased from Biodesign Int. Kennebunk, ME. The 3G8-FITC anti-Fc γ RIII and FITC-mouse monoclonal antibody to the human CD23 receptors used in flow cytometric studies were purchased from Medarex, Inc., Annandale, NJ and Calteg, San Francisco, CA, respectively. The anti-CD23 monoclonal blocking antibody (mAb135) is described in Mossalayi et al. (1992, EMBO J. 11:4323-4328). The Fc ϵ RII (CD23) antibody and F(ab')₂-FITC fragments used in the immunofluorescence studies were purchased from Serotec Ltd., Oxford, UK. The immortalized B-cell line 8.1.6, is described in Weenink et al. (1977, International Immunol. 9(6):889-896). The Fc γ -RI, -RII -RIII and Fc ϵ RII cDNA probes are described in McKenzie (1994, Current Opinion in Hematol. 1:45-52). All drug concentrations are expressed as final bath concentrations. Isoproterenol and ACh were made fresh for each experiment, dissolved

in normal saline to prepare 10^{-4} M and 10^{-3} M solutions, respectively. The human tissue was provided by the Cooperative Human Tissue Network which is funded by the National Cancer Institute.

The Results of the experiments presented herein are now described.

5 Role of Fc receptors in altered responsiveness of atopic asthmatic sensitized airway smooth muscle

Passive sensitization of isolated naive airway smooth muscle (ASM) tissue with human atopic asthmatic serum induces changes in the tissue's agonist-mediated constrictor and relaxant responsiveness that phenotypically resemble the pro-asthmatic state (Hakonarson et al., 1995, Am. J. Physiol. (Lung Cell Mol. Physiol.) 269:L645-L652). To examine whether these effects of atopic asthmatic serum are mediated, at least in part, by the presence of elevated levels of IgE in the sensitizing serum, constrictor and relaxation responses were separately examined in TSM segments that were treated with human control or atopic asthmatic serum in the absence and presence of a blockade of specific Fc receptors, or following depletion of immune complexes in the sensitizing serum by treatment with Sepharose Staphylococcus protein A (SpA) (Kessler, 1975, J. Immunol. 115:1617-1624). As shown in Figure 1, relative to tissues incubated with control serum (open circles), the maximal constrictor (Tmax) responses to ACh were significantly enhanced in TSM passively sensitized with atopic asthmatic serum (filled circles). Accordingly, the mean \pm SE Tmax values amounted to 121.7 ± 5.3 and 146.0 ± 15.5 g/g TSM wt. in the control and sensitized tissues, respectively ($p < 0.01$). The induced augmented constrictor responses to ACh, however, were largely prevented in atopic serum-sensitized tissues that were pre-treated with an anti-CD23 monoclonal blocking antibody (anti-CD23 MAb) (Figure 1A; open squares) or when the sensitizing serum was depleted of its immune complexes by pre-treatment with SpA (Figure 1B; open squares).

In separate studies, during comparable levels of initial sustained ACh-induced contractions in atopic asthmatic sensitized and control serum-treated airway

segments, averaging 38 and 43% of Tmax, respectively, administration of the beta-adrenergic receptor agonist, isoproterenol, elicited cumulative dose-dependent relaxation of the pre-contracted TSM segments (Figure 2). Relative to control TSM, the maximal relaxation responses (Rmax) and sensitivities (pD₅₀; i.e., -log ED₅₀) to isoproterenol were significantly attenuated in the atopic asthmatic serum-sensitized TSM. Accordingly, the mean Rmax values for isoproterenol amounted to 26.0±5.1 % in the atopic sensitized TSM, compared to 47.0±4.7 % in the control serum-treated TSM (p<0.005), with corresponding pD₅₀ values averaging 5.82±0.16 and 6.59±0.11 -log M, respectively (p<0.005). However, the attenuated isoproterenol-induced relaxation responses were ablated in atopic serum-sensitized TSM that were pretreated with anti-CD23 MAb (Figure 2A), or when the sensitizing serum was initially depleted of its immune complexes with SpA (Figure 2B).

In contrast to the above observations obtained in atopic asthmatic serum-sensitized TSM, in tissues incubated with control serum, neither anti-CD23 MAb nor pretreatment of the control serum with SpA affected the subsequent contractility of the tissue to ACh or relaxation responsiveness to isoproterenol. Moreover, contrasting the above inhibitory effects of anti-CD23 MAb in atopic sensitized TSM, exposure of the sensitized tissues to a monoclonal blocking antibody to the FcγRIII receptor, which is also expressed in ASM (see below), had no appreciable effect on the heightened constrictor responsiveness of the tissue to ACh or attenuated relaxation to isoproterenol.

Effects of IgE and IgE immune complexes on ASM responsiveness.

In light of the above observations suggesting a role for an IgE immune complex/FcεRII receptor interaction in mediating altered ASM responsiveness in the atopic asthmatic sensitized state, the issue of whether the sensitizing effects of the atopic asthmatic serum could be simulated by treatment of naive TSM with either exogenous IgE or IgE immune complexes was examined. Constrictor and relaxant dose-response relationships to ACh and isoproterenol, respectively, were separately compared in TSM exposed for 24 hours to vehicle alone (control) or varying

concentrations of IgE or IgE immune complexes. Relative to their respective controls, the maximal constrictor responses to ACh were significantly ($p < 0.05$) enhanced in tissues treated for 24 hours with an optimal concentration of IgE immune complexes (i.e., 15:5 $\mu\text{g/ml}$ of IgE/anti-IgE), wherein the mean \pm T_{max} responses amounted to 108.8 \pm 10.2 vs 94.2 \pm 5.1 g/g TSM wt obtained in the control tissues (Figure 3). Comparably, during similar initial sustained levels of ACh-induced contractions (i.e., ~45% T_{max}), the subsequent relaxation responses to cumulative administration of isoproterenol were markedly reduced in the IgE immune complex-treated vs. control tissues (Figures 4), wherein the R_{max} responses amounted to 36.8 \pm 10.4 vs. 67.8 \pm 6.0 %, respectively ($p < 0.05$), and the corresponding pD₅₀ values averaged 6.70 \pm 0.08 and 6.84 \pm 0.11 -log M, respectively ($p < 0.05$). Qualitatively, similar results were obtained in tissues treated with IgE alone, although the changes in ACh-induced contraction and attenuation of isoproterenol-induced relaxation in these tissues were quantitatively less pronounced than those obtained in IgE immune complex-treated TSM.

Fc receptor expression in atopic asthmatic sensitized airway smooth muscle

In extending the above pharmacodynamic studies, in separate experiments, the issues of whether cultured ASM cells and isolated ASM tissue express Fc receptors and whether the expression of these receptors is modulated in the atopic asthmatic sensitized state was examined. Using RT-PCR and Fc receptor specific primers for the different Fc γ and Fc ϵ receptor subtypes, cDNA was reverse transcribed from total isolated RNA primed with random hexamer primers, and Southern blots were probed with human cDNA probes specific for individual Fc γ and Fc ϵ receptor genes as described in the Methods section. A 415-bp α -actin probe was also used to control for gel loading, and the signals for the Fc γ , Fc ϵ and α -actin PCR products were quantitated on a Phosphorimager.

In contrast to the lack of detectable expression of Fc γ RI and Fc γ RII specific mRNA, ASM cells expressed mRNA specific for the Fc γ RIII receptor (Figure 5A). In parallel with the unaltered constitutive expression of α -actin mRNA, the

FcγRIII signal did not temporally vary in cells treated for up to 24 hours with either control or atopic sensitized serum, and there were no significant differences in FcγRIII mRNA expression between control and sensitized cells. Expression of the high affinity IgE receptor, FcεRI, was also undetectable in the ASM cells. On the other hand, as shown in Figure 5B, expression of mRNA specific for the low affinity IgE receptor, FcεRII (i.e., CD23), was detected in both the control and atopic asthmatic sensitized cells. Moreover, in contrast to control serum-treated ASM cells wherein the mRNA signal did not systematically vary with time, the intensity of the FcεRII mRNA signal progressively increased at 6 and 24 hours in the atopic asthmatic serum-sensitized cells. Thus, rabbit ASM cells expressed mRNA specific for both the low-affinity FcγRIII and FcεRII receptors as verified by the presence of 254 bp (FcγRIII) and 333 bp (FcεRII) cDNA fragments. In contrast to FcγRIII, expression of FcεRII was significantly upregulated at 24 hr (i.e., > 2 fold) following treatment with atopic asthmatic serum.

Similar results were obtained in comparable experiments conducted on isolated human tracheal smooth muscle tissue wherein adjacent alternating sections were exposed for 24 hours to either control or atopic asthmatic serum. Following RNA extraction, PCR products generated using oligo (dT)-primed human cDNA and the above human-specific FcγRIII and FcεRII primers, as well as a primer for the constitutively expressed ribosomal protein, RPL7, were loaded in separate lanes on a 1.2% agarose gel. The Southern blots were then analyzed using ³²P-labeled human-specific FcγRIII, FcεRII, and RPL7 (157-bp) probes. As depicted in Figure 6, following 24 hours of incubation with serum, the signals for expression of FcγRIII and RPL7 mRNA were similar in the control (CO) and atopic asthmatic-sensitized (SE) tissue samples (Figure 6A). In contrast, the signal for FcεRII mRNA expression, although detectable in the control samples, was markedly induced in the atopic asthmatic serum-treated tissues (Figure 6B), whereas that of RPL7 was unaffected in these cells. Thus, a marked induction of FcεRII expression was observed in the atopic

asthmatic serum-sensitized sample (i.e., > 7.5 fold), whereas expression of FcγRIII or RPL7 was similar in both samples.

In light of the above observations, together with the preceding pharmacodynamic findings implicating a role for IgE immune complex/CD23 interaction in mediating the changes in agonist responsiveness in the atopic asthmatic sensitized tissues (Figures 1 and 2), the issue of whether the observed upregulation of FcεRII expression in the atopic asthmatic sensitized state was attributable to activation of the FcεRII receptor was examined. As shown in Figure 7, using ASM tissue sections isolated from the same human lung specimen it was discovered that, whereas the tissue samples exposed to atopic asthmatic serum (SE) displayed markedly induced FcεRII expression relative to control (CO) serum-treated samples, the upregulation of FcεRII expression was largely inhibited by pretreatment of the atopic serum-sensitized tissues with anti-CD23 monoclonal antibody (SE*). Thus, anti-CD23 MAb significantly attenuated the induction of FcεRII mRNA expression at 24 hours in atopic asthmatic-serum sensitized airway smooth muscle. Moreover, in separate complimentary experiments examining the effects of exogenous administration of IgE immune complexes on FcεRII expression in cultured ASM cells, it was found that treatment of the cells with IgE immune complexes induced progressive enhancement of FcεRII mRNA expression (Figure 8A), resulting in a near 5-fold increase in FcεRII expression at 24 hours (Figure 8B). Additionally, in concert with the above ASM tissue studies, pretreatment of cells with anti-CD23 monoclonal antibody significantly inhibited (i.e., by ~40%) the magnitude of the IgE immune complex-induced FcεRII expression at 24 hours (Figure 8B). Thus, a progressive induction of FcεRII mRNA expression up to 24 hours following treatment with IgE immune complexes, and inhibition of FcεRII expression at 24 hr in the presence of anti-CD23 MAb (hatched bar) was observed.

Taken together, the above observations demonstrate that rabbit and human ASM express mRNA specific for the low-affinity FcγRIII and FcεRII receptors for IgG and IgE, respectively, and that the expression of the FcεRII receptor mRNA, as

examined using RT-PCR, is upregulated by treatment with either human atopic asthmatic serum or exogenously administered IgE immune complexes, secondary to activation of the endogenously expressed FcεRII receptor.

Expression of FcγRIII and FcεRII cell surface proteins in sensitized
airway smooth muscle

Given the above findings, the issue of whether ASM cells express FcγRIII and FcεRII receptor proteins on their cell surface was examined by flow cytometry. As shown in Figure 9, ASM cells expressed both the low-affinity FcγRIII (Figure 9A) and FcεRII (Figure 9B) receptors as surface protein. In contrast to unaltered FcγRIII receptor expression after exposure of the cells for 24 hours to control or atopic asthmatic serum (Figure 9A), cell surface expression of the FcεRII receptor was increased by > 2-fold in the atopic asthmatic serum-treated vs. control serum-treated cells (Figure 9B). Thus, rabbit ASM cells expressed surface protein specific for both FcγRIII and FcεRII receptors. In contrast to FcγRIII receptor expression which was unaltered in the presence of atopic asthmatic serum, expression of the FcεRII receptor was increased by > two-fold (i.e., from 17 to 36% in the presence of atopic asthmatic serum).

To further substantiate the above observations, sensitized ASM cells and tissue were subsequently examined for evidence of altered FcεRII receptor expression in by immunofluorescence staining. As shown in Figure 10, relative to an isotype negative control antibody (upper panels: A and B), FcεRII staining with an anti-CD23 antibody was weakly positive both in control (CO) serum-treated cells and tissue (lower left panels: A and B). Moreover, in parallel with the above results based on flow cytometric analysis, surface staining for the CD23 receptor protein was notably enhanced in the atopic asthmatic serum-treated cells and tissue (Figure 10; lower right panels: A and B). Thus, significantly enhanced FcεRII (CD23) receptor staining in ASM cells and tissue treated with atopic asthmatic serum (i.e., lower SE panels in Panel A and Panel B) was observed.

Previous studies have implicated both mast cells and other pro-inflammatory cells in the pathogenesis of asthma. The present study addressed the hypothesis that the resident airway smooth muscle itself also plays an etiologic role in the pathobiology of the disease. The results provide compelling new evidence demonstrating that: 1) ASM cells express mRNA and surface protein for the low-affinity FcγRIII (CD16) and FcεRII (CD23) receptors for IgG and IgE, respectively; 2) in contrast to the FcγRIII receptor, expression of the FcεRII receptor is significantly upregulated following treatment with either atopic asthmatic serum or IgE immune complexes, a finding similar to that obtained in a passively sensitized human ASM; 3) the induced changes in FcεRII receptor expression are associated with altered ASM responsiveness to muscarinic/cholinergic and β-adrenoceptor activation in the atopic asthmatic sensitized state; and 4) the latter changes in FcεRII receptor expression and responsiveness in atopic asthmatic sensitized ASM can be attributed to activation of intrinsically expressed FcεRII receptors by immune complexed IgE.

The use of passive sensitization of rabbit and human isolated airways with human atopic asthmatic serum provides a practical *in vitro* experimental approach to examine the regulation of airway responsiveness in the atopic asthmatic state (Hakonarson et al., 1997, J. Clin. Invest. 99:117-124; Hakonarson et al., 1995, Am. J. Physiol. (Lung Cell Mol. Physiol.) 269:L645-L652). In this connection, the observed changes in responsiveness in the atopic sensitized tissues closely mimicked the perturbations in airway function that characterize the *in vivo* asthmatic condition, including exaggerated bronchoconstrictor responsiveness to contractile agonists and impaired airway relaxation to β-adrenoceptor stimulation (Hakonarson et al., 1997, J. Clin. Invest. 99:117-124; Hakonarson et al., 1995, Am. J. Physiol. (Lung Cell Mol. Physiol.) 269:L645-L652). When the mechanistic link between exposure of naive airway tissue to atopic asthmatic serum and its resultant altered responsiveness was previously addressed using the same experimental model described herein, it was observed that the latter effect was largely mediated by the induced autologous expression and autocrine action of the cytokine, IL-1β, in atopic sensitized ASM

(Hakonarson et al., 1997, J. Clin. Invest. 99:117-124). Moreover, the mechanism of action of IL-1 β in eliciting the altered responsiveness in sensitized tissues was attributed to its induced upregulated expression of G_i protein, specifically Gi α_2 and Gi α_3 which inhibit intracellular cAMP accumulation (Hakonarson et al., 1996, J. Clin. Invest. 97:2593-2600).

The data presented herein establish that the sequence of events leading to cytokine-induced changes in ASM responsiveness in the sensitized state is initiated by IgE-coupled Fc ϵ RII receptor activation involving the ASM itself. The present observations demonstrate that either depletion of serum immune complexes or pretreatment with a specific anti-CD23 monoclonal blocking antibody largely prevented the changes in ASM responsiveness induced by exposure of the tissues to atopic asthmatic serum (Figures 1 and 2). Furthermore, in complimentary studies, exposure of naive ASM tissue to IgE immune complexes produced changes in ASM responsiveness (Figures 3 and 4) which closely resembled those observed in the atopic sensitized state. Thus, IgE-coupled Fc receptor activation principally involving the Fc ϵ RII receptor in ASM itself appears to be fundamentally responsible for producing the perturbations in airway responsiveness that characterize the pro-asthmatic state.

In concert with the above pharmacodynamic results, additional experiments demonstrated the presence of constitutive Fc ϵ RII and Fc γ RIII receptor mRNA and cell surface expression, both in cultured ASM cells and human ASM tissue (Figures 5 and 6). Furthermore, in contrast to Fc γ RIII expression, which was unaltered in the presence of atopic asthmatic serum, expression of Fc ϵ RII was markedly enhanced in ASM exposed to the sensitizing atopic serum (Figures 5 and 6). In considering these results, it is relevant to note that a number of studies have reported a direct correlation between serum IgE levels and Fc ϵ RII and -RI expression (Spiegelberg et al., 1981, J. Clin. Invest. 68:845-852; Conroy et al., 1977, J. Immunol. 118:1317-1321; Malveaux et al., 1978, J. Clin. Invest. 62:176-181). While these reports raised the notion of a possible common mechanism regulating the production of both IgE and its receptors, more recent evidence based on studies using different cell

lines suggests that IgE itself may enhance FcεRI (Lantz et al., 1997, J. Immunol. 158:2517-2521; Yamaguchi et al., 1997, J. Exp. Med. 185(4):663-672) or FcεRII expression (Lee et al., 1987, J. Immunol. 139:1191-1198; Daeron et al., 1986, J. Immunol. 136:1612-1619; Lee et al., 1986, J. Immunol. 136:4573-4580). The latter
5 concept is supported by the present experiments, wherein it was observed that the induced expression of FcεRII in ASM treated with atopic asthmatic serum was largely inhibited in the presence of an anti-CD23 blocking antibody (Figure 7). Moreover, as further support for the concept of IgE-dependent modulation of FcεRII expression, it was also observed that exogenously administered IgE immune complexes elicited an
10 increase in FcεRII expression (Figure 8A) and, further, that this effect was significantly inhibited by pretreatment with anti-CD23 MAbs (Figure 8B). Thus, the data presented herein are consistent with the presence of a positive feedback system in ASM wherein IgE upregulates the expression of its own low affinity receptor. This notion is in general agreement with previous studies which reported the presence of IgE-dependent
15 Fcε receptor induction in other cell types (Lantz et al., 1997, J. Immunol. 158:2517-2521; Yamaguchi et al., 1997, J. Exp. Med. ??:663-672; Lee et al., 1987, J. Immunol. 139:1191-1198; Daeron et al., 1986, J. Immunol. 136:1612-1619; Lee et al., 1986, J. Immunol. 136:4573-4580). However, in these earlier studies mRNA levels for Fcε receptor expression were not examined, and the increased cell surface expression of the
20 receptor was attributed to IgE-mediated inhibition of proteolytic cleavage of the receptor from its membrane binding site (Lee et al., 1987, J. Immunol. 139:1191-1198). Recognizing the limitations in quantitative analysis using RT-PCR, the present observations demonstrating the presence of IgE-dependent enhanced expression of FcεRII mRNA suggest that IgE exerts its upregulatory action on FcεRII expression, at
25 least in part, via a pre-translational effect.

In recent years, the complexity of transmembrane signaling via the FcεRII receptor has been partially unraveled. In this regard, together with IgE binding, signaling via the FcεRII receptor is known to include coupling of the receptor to complement receptor 2 (CR2), also referred to as CD21, which is a receptor for

fragments of the complement component, C3 (Aubry et al., 1992, *Nature* 358:505-507). Further, as demonstrated in B-lymphocytes, CR2 co-ligates with the membrane protein, CD19 and, in the presence of C3 fragments bound to antigen/immune complexes (e.g., IgE/IgG and/or IgM), this membrane-linked network of molecules acts synergistically to mediate the cytokine activities of FcεRII (Sutton et al., 1993, *Nature* 366:421-428; Delespesse et al., 1992, *Immunol. Rev.* 125:77-97; Matsumoto et al., 1991, *J. Exp. Med.* 173:55-64). Accordingly, in B-lymphocytes, this antigen/IgE immune complex network coupled to the FcεRII receptor apparently serves to facilitate antigen presentation to antigen-specific CD4+ (helper) T-lymphocytes, a process resulting in the expression of the CD4+/Th2 phenotype. The latter phenomenon is associated with the release of such Th2-derived cytokines as IL-4 and IL-5 which are involved in B-lymphocyte switching to IgE production and eosinophil accumulation, respectively (Delespesse et al., 1992, *Immunol. Rev.* 125:77-97). With potential implications related to asthma, recent evidence based on studies conducted in house dust mite-immunized mice suggests that the above mechanism of IgE/FcεRII-coupled facilitation of antigen presentation to T-lymphocytes is important for both the induction of the Th2-type immune response in the lungs and the subsequent infiltration of eosinophils into the airways following inhaled antigen challenge (Coyle et al., 1996, *J. Exp. Med.* 183:1303-1310).

In light of the above evidence regarding the role of CD23 receptor activation in eliciting Th-2 cytokine production and pulmonary eosinophilic infiltration in the antigen-sensitized state, the results of the present study raise the consideration that, apart from inducing changes in airway responsiveness, upregulated FcεRII expression and activation in airway smooth muscle cells may also play a role in modulating local airway immune responses. Indeed, this compelling concept is in part supported by the recent observation that airway smooth muscle cells are autologously induced to release IL-1β in the atopic asthmatic sensitized state (Hakonarson et al., 1997, *J. Clin. Invest.* 99:117-124), a finding which, together with the present observations, suggests the presence of FcεRII-coupled local stimulation of cytokine

release and signaling. In this context, it is important to determine whether IgE-coupled activation of FcεRII receptors in airway smooth muscle can also induce the release of Th-2-like cytokines and, thereby, promote local infiltration of the airways with eosinophils and other proinflammatory cells seen in the asthmatic state.

5 In conclusion, the present study has identified the presence and up-regulated expression of FcεRII receptors in atopic asthmatic-sensitized airway smooth muscle, and has further demonstrated that the latter phenomenon is associated with the induction of altered airway responsiveness in the atopic asthmatic sensitized state.

10 While comparably up-regulated expression of the FcεRII receptor has been demonstrated in different bone marrow-derived circulating leukocytes in atopic asthmatic individuals (Williams et al., 1992, J. Immunol. 149:2823-2829; Gagro et al., 1993, Int. Arch. Allergy Immunol. 101:203-208; Rabatic et al., 1993, Exp. Immunol. 94:337-340; Joseph et al., 1983, J. Clin. Invest. 71:221-230), the present observations raise a novel consideration regarding the pathobiology of asthma.

15 Accordingly, given the important immunological and proinflammatory responses attributed to FcεRII receptor activation, the present findings identify an important role and mechanism for the resident airway smooth muscle in autologously inducing its own IgE-mediated state of altered responsiveness contributing to the atopic asthmatic condition.

20 The disclosures of each and every patent, patent application and publication cited herein are hereby incorporated herein by reference in their entirety.

25 While this invention has been disclosed with reference to specific embodiments, it is apparent that other embodiments and variations of this invention may be devised by others skilled in the art without departing from the true spirit and scope of the invention. The appended claims are intended to be construed to include all such embodiments and equivalent variations.

What is claimed is:

1. A method of preventing induction of an asthmatic state in a human patient comprising administering to said human an anti-FcεRII receptor protein ligand suspended in a pharmaceutically acceptable carrier in an amount sufficient to inhibit binding of IgE to an anti-FcεRII receptor protein thereby preventing induction of said asthmatic state in said human.
2. The method of claim 1, wherein said ligand is selected from the group consisting of an isolated protein, an isolated polypeptide and a non-peptide.
3. The method of claim 2, wherein said ligand is selected from the group consisting of a polyclonal antibody, a monoclonal antibody, a synthetic antibody, a chimeric antibody and a humanized antibody.
4. The method of claim 3, wherein said anti-FcεRII receptor protein ligand is an anti-FcεRII receptor protein antibody.
5. The method of claim 1, wherein said ligand is administered to said human in the form of an isolated DNA encoding and capable of expressing said ligand.
6. The method of claim 5, wherein said DNA is formulated in a viral or a non-viral vector.
7. The method of claim 6, wherein said viral vector is selected from the group consisting of a recombinant vaccinia virus, a recombinant adenovirus, a recombinant retrovirus, a recombinant adeno-associated virus and a recombinant avian pox virus.

8. The method of claim 7, wherein said non-viral vector is selected from the group consisting of a liposome and a polyamine conjugated DNA.
9. The method of claim 1, wherein said anti-FcεRII receptor protein ligand is administered to the human in an amount between about 1 ng/kg and about 100 mg/kg of patient body weight.
10. The method of claim 1, wherein said pharmaceutically acceptable carrier is physiological saline.
11. A method of treating asthma in a human patient comprising administering to said human an anti-FcεRII receptor protein ligand suspended in a pharmaceutically acceptable carrier in an amount sufficient to inhibit binding of IgE to an anti-FcεRII receptor protein thereby treating asthma in said human.
12. The method of claim 11, wherein said ligand is selected from the group consisting of an isolated protein, an isolated polypeptide and a non-peptide.
13. The method of claim 12, wherein said ligand is selected from the group consisting of a polyclonal antibody, a monoclonal antibody, a synthetic antibody, a chimeric antibody and a humanized antibody.
14. The method of claim 13, wherein said anti-FcεRII receptor protein ligand is an anti-FcεRII receptor protein antibody.
15. The method of claim 11, wherein said ligand is administered to said human in the form of an isolated DNA encoding and capable of expressing said ligand.

16. The method of claim 15, wherein said DNA is formulated in a viral or a non-viral vector.

17. The method of claim 16, wherein said viral vector is selected from the group consisting of a recombinant vaccinia virus, a recombinant adenovirus, a recombinant retrovirus, a recombinant adeno-associated virus and a recombinant avian pox virus.

18. The method of claim 17, wherein said non-viral vector is selected from the group consisting of a liposome and a polyamine conjugated DNA.

19. The method of claim 11, wherein said anti-FcεRII receptor protein ligand is administered to the human in an amount between about 1 ng/kg and about 100 mg/kg of patient body weight.

20. The method of claim 21, wherein said pharmaceutically acceptable carrier is physiological saline.

21. A method of treating a human having asthma comprising administering to said human a pharmaceutically effective amount of an isolated nucleic acid encoding an anti-FcεRII receptor protein ligand, wherein said nucleic acid expresses said ligand in vivo in an amount sufficient to treat said asthma.

22. A method of identifying an anti-FcεRII receptor protein ligand comprising

providing a mixture comprising IgE and a population of cells which express an FcεRII receptor protein,

incubating said mixture in the presence or absence of a test ligand, and

measuring the level of IgE bound to said FcεRII receptor protein, wherein a lower level of IgE bound to said cells in the presence of said test compound compared with the level of binding of IgE to said cells in the absence of said test compound is an indication that said test compound is an anti-FcεRII receptor protein ligand.

23. The method of claim 22, wherein said cells are airway smooth muscle cells.

24. A ligand useful for inhibiting binding of IgE to an FcεRII receptor protein identified using the method of claim 22.

25. A method of inhibiting binding of IgE to an FcεRII receptor protein expressed on a cell comprising administering to said cell an anti-FcεRII receptor protein ligand.

ABSTRACT OF THE INVENTION

Methods of treating or preventing induction of asthma in a human patient are provided. The methods comprise administering to the human an anti-FcεRII receptor protein ligand suspended in a pharmaceutically acceptable carrier in an amount sufficient to inhibit binding of IgE to an anti-FcεRII receptor protein thereby treating or preventing induction of asthma in the human.

Figure 1.

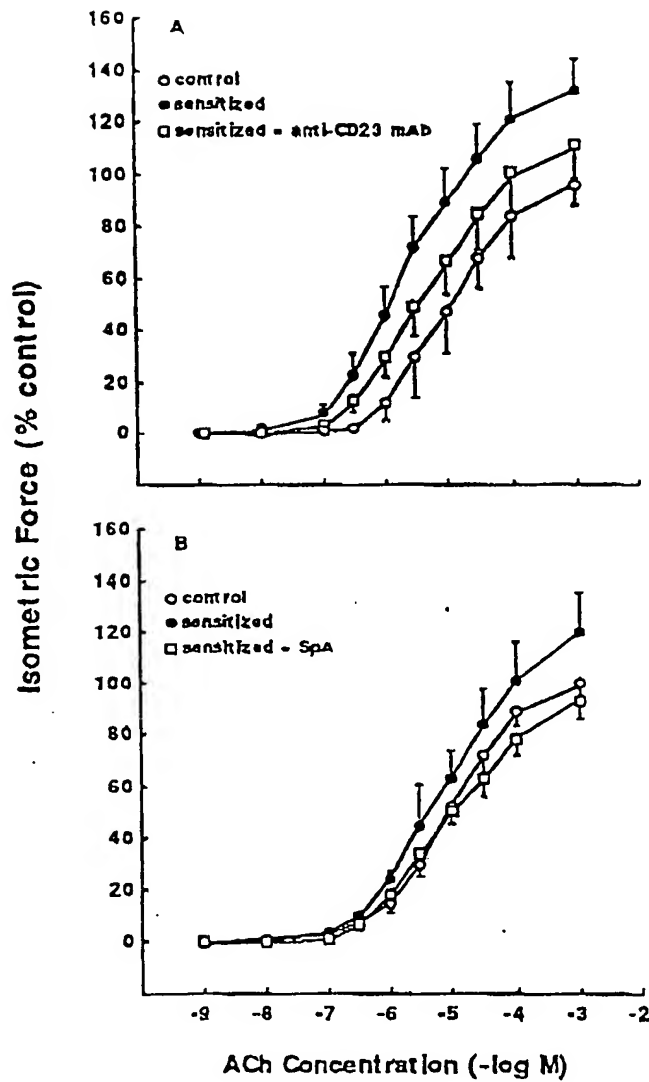
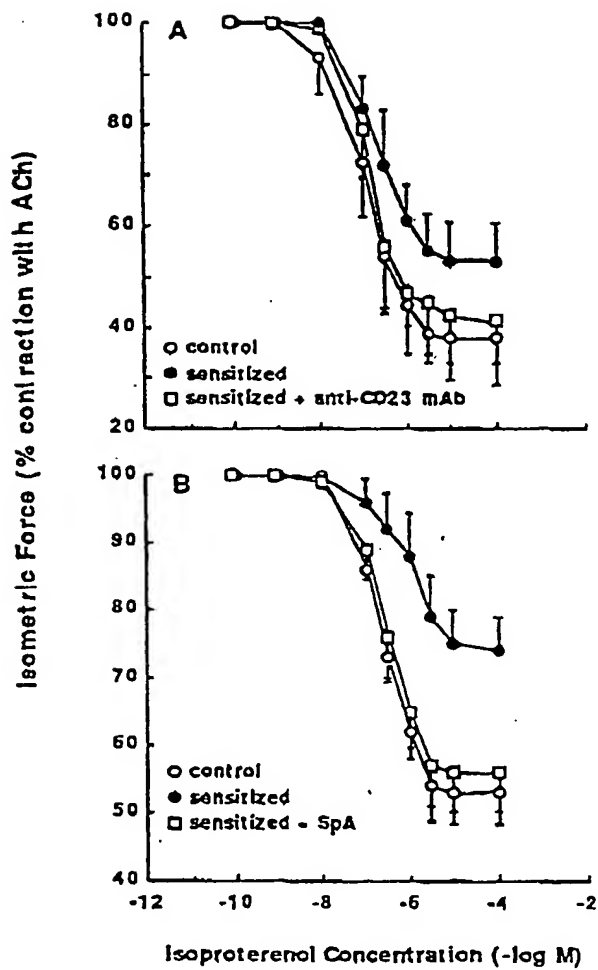


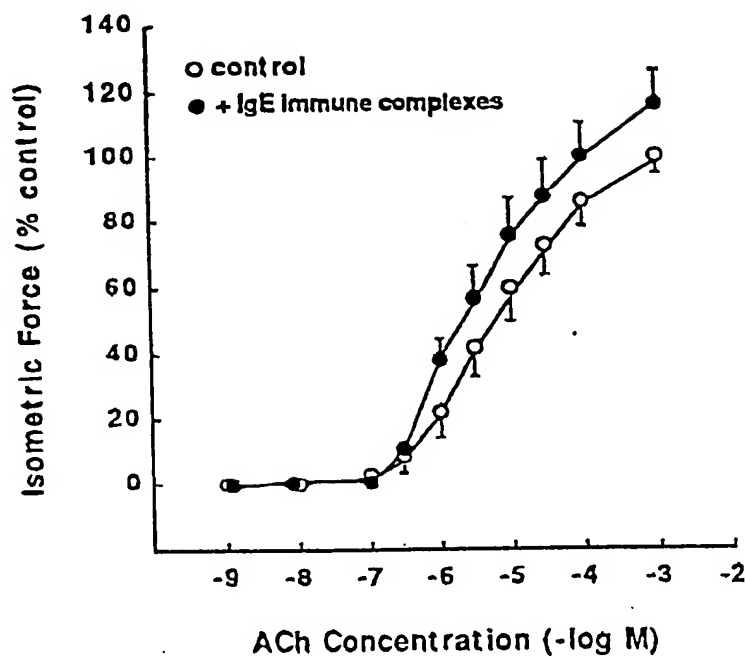
Figure 2.

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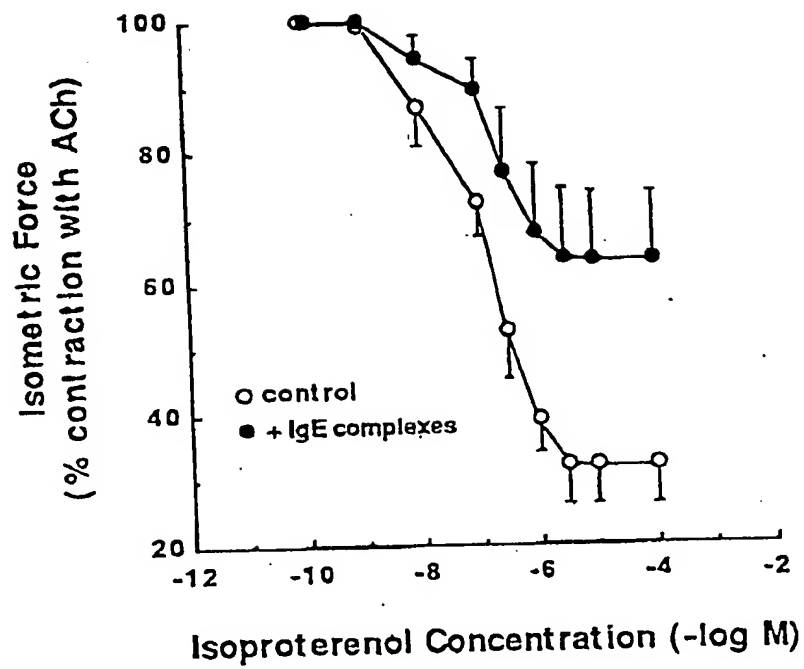
HANSEN & EMMETT

Figure 3



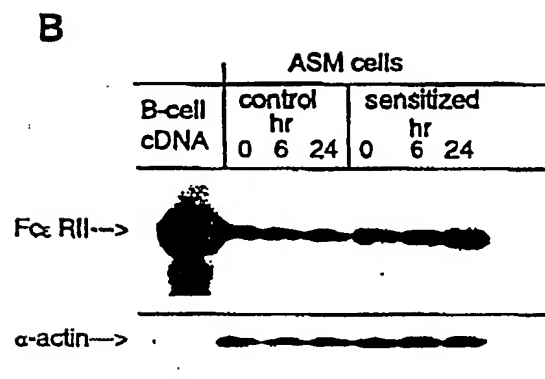
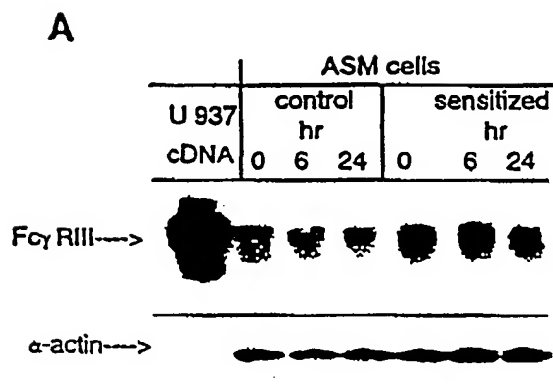
HANSON & GUNDEL

Figure 4.



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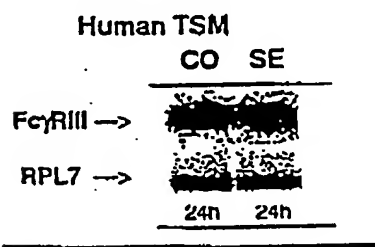
Figure 5.



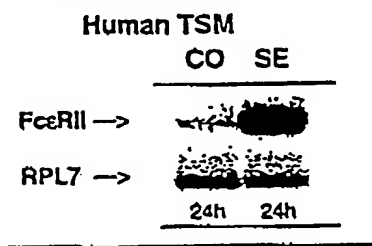
HANSON & GUNTER

Figure 6

A.

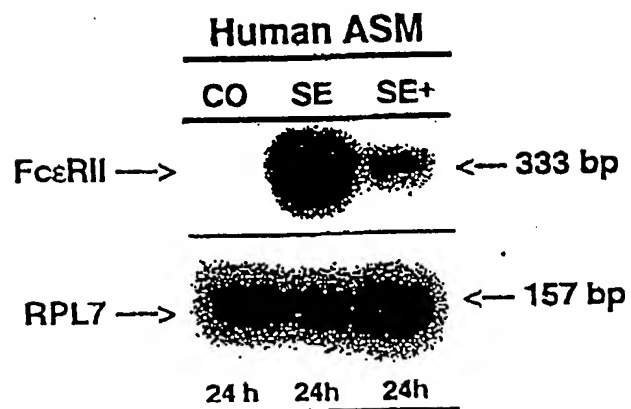


B.



Hammann & Bernstein

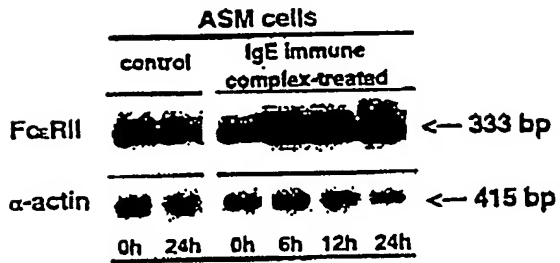
Figure 7.



HANONSON & SPURSTEIN

Figure 8

A



B

